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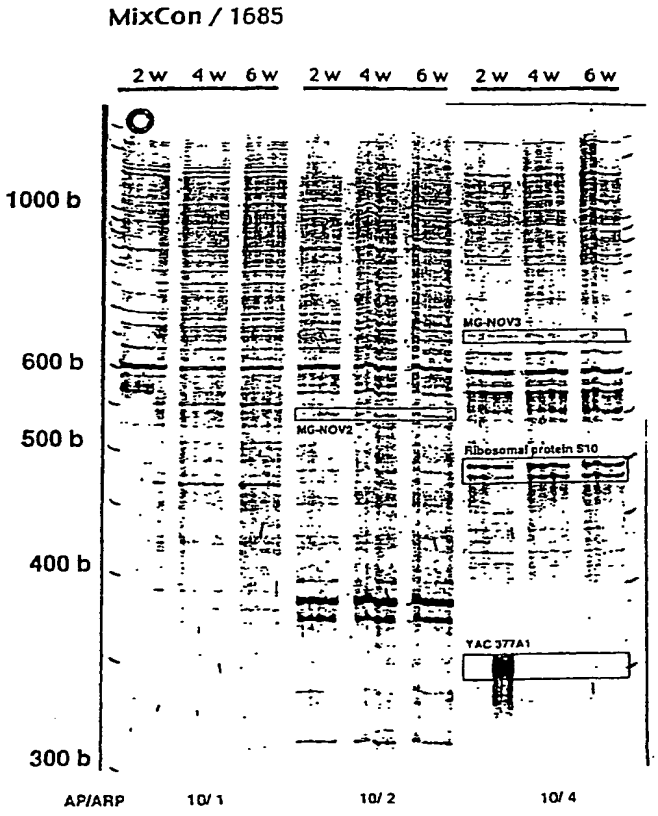
(54) Title: DIFFERENTIAL EXPRESSION OF ORGANELLAR GENE PRODUCTS

(57) Abstract

Compositions and methods are provided for identifying factors, including organellar factors, that are differentially expressed when cells in different states, such as metabolic, respiratory, disease or apoptotic states, are compared. In preferred embodiments the invention relates to mitochondria DNA depleted (ρ^0) and cytoplasmic hybrid (cybrid) cells, such as mitochondrial cybrid cells. Use of the invention to identify species specific expression of organellar factors such as organelle associated macromolecules is contemplated. Also disclosed are examples of organellar factors that are differentially expressed in organelle associated disease, including a variety of human genes that are differentially expressed in Alzheimer's disease.



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DIFFERENTIAL EXPRESSION OF ORGANELLAR GENE PRODUCTS

TECHNICAL FIELD

The invention relates to factors encoded by genes that are differentially expressed in cellular models of particular disease states associated with organelles in cells as compared to control cells, or in cells response to various compounds or conditions thought to influence organellar function. Differentially expressed genes and factors in organelle-associated diseases include organellar factors, *i.e.*, macromolecules found within or associated with organelles, and cellular factors that negatively or positively influence, either directly or indirectly, the amount and/or activity of such macromolecules. Organellar factors include nucleic acids and proteins that are expressed from genes that are derived from a cell's or organism's nuclear genome, as well as those expressed from the genomes of organelles such as mitochondria or chloroplasts. Cells and cellular models useful in the invention include cybrids and rho-zero (ρ^0) cells. Cybrids are cellular hybrids having a nucleus derived from a first cell line and a cytoplasmic component (which may include organelles) derived from a second cell line or from an organism suffering from, or suspected of being prone to develop, a disease or disorder. Rho^0 cells are cells derived from an organism or from cell lines that have been treated so as to eliminate the genomes of their mitochondria and/or chloroplasts. Differential expression can reflect a comparison between ρ^0 and control cells; between cybrids and control cells; between cells, including cybrids and ρ^0 cells, that have been exposed to one or more stressors.

BACKGROUND OF THE INVENTION

The cell is the basic unit of life and comprises a variety of subcellular compartments including, *e.g.*, organelles. An organelle is a structural component of a cell that is physically separated, typically by one or more membranes, from other cellular components, and which carries out specialized cellular functions.

Mitochondria and chloroplasts are two organelles of particular interest with regard to the present invention as each contains its own DNA genome. These organellar genomes encode a fraction of the gene products required for organellar function, the remainder of such gene products being encoded by the nuclear genome. Relatively little is known about the mechanisms by which mitochondrial and chloroplast gene products, which may be encoded by nuclear sequences or sequences found in the respective organellar genomes, are coordinately regulated (Surpin and Chory, *Essays Biochem. 32*:113-125, 1997).

Because of the role of mitochondria in various diseases and disorders, there is a need to identify genetic sequences, present in either the nuclear or mitochondrial genomes (or both), that encode mitochondrial gene products and that are differentially expressed in such diseases and disorders. There is also a need for nucleic acids comprising such genetic sequences that can be used as probes in diagnostic, prognostic and pharmacogenomic assays, useful in the therapeutic management of such diseases and disorders. Such nucleic acids can also be used to produce gene products that can be used as novel targets in methods for identifying therapeutic compounds, including high through-put screening, useful to treat such diseases and disorders.

Additionally, in view of the economic desirability of enhanced crop production, and the role of chloroplasts in processes such as photosynthesis that are essential for producing biomass, there is a need to identify genetic sequences present in the nuclear or chloroplast genomes (or both), that encode chloroplast gene products that are differentially expressed under different environmental conditions or in response to extraneously added agents. Such nucleic acids can be used to identify and produce gene products that may be used as novel targets in methods for identifying compounds and conditions that promote or optimize photosynthesis and other biomass producing processes.

A number of difficulties are also associated with killing eukaryotic pathogens and parasites without harming their eukaryotic hosts, such that species-to-species variation in organellar functions may be exploited to develop novel antibiotics. There is thus a need to identify genetic sequences encoding organellar functions that are

differentially expressed in a species-specific fashion in response to compounds, particularly compounds that are known or candidate antibiotics that kill or slow the growth of eukaryotic pathogens and parasites without harming their eukaryotic hosts. Such nucleic acids can be used to identify and produce gene products that may be used as novels targets in methods for identifying antibiotics, including high throughout screening, useful to treat diseases and disorders resulting from such eukaryotic pathogens and parasites.

The present invention fulfills these and other needs. These and other advantages of the present invention will become more apparent by the detailed description of the invention provided herein.

Mitochondria

The organelle known as the mitochondrion (plural, mitochondria) is the main energy source in cells of higher organisms. Mitochondria provide direct and indirect biochemical regulation of a wide array of cellular respiratory, oxidative and metabolic processes. These include electron transport chain (ETC) activity, which drives oxidative phosphorylation to produce metabolic energy in the form of adenosine triphosphate (ATP), and which also underlies a central mitochondrial role in intracellular calcium homeostasis. In addition to their role in energy production in growing cells, mitochondria (or, at least, mitochondrial components) participate in programmed cell death (PCD), also known as apoptosis (Newmeyer et al., *Cell 79*:353-364, 1994; Liu et al., *Cell 86*:147-157, 1996; for general reviews of apoptosis, and the role of mitochondria therein, see Green and Reed (*Science 281*:1309-1312, 1998), Green (*Cell 94*:695-698, 1998) and Kromer (*Nature Medicine 3*:614-620, 1997).

Mitochondrial ultrastructural characterization reveals the presence of an outer mitochondrial membrane that serves as an interface between the organelle and the cytosol, a highly folded inner mitochondrial membrane that appears to form attachments to the outer membrane at multiple sites, and an intermembrane space between the two mitochondrial membranes. The subcompartment within the inner mitochondrial membrane is commonly referred to as the mitochondrial matrix. (For a review, *see,*

e.g., Ernster and Schatz, *J. Cell Biol. 91*:227s-255s, 1981.) The cristae, originally postulated to occur as infoldings of the inner mitochondrial membrane, have recently been characterized using three-dimensional electron tomography as also including tube-like conduits that may form networks, and that can be connected to the inner membrane by open, circular junctions (Perkins et al., *Journal of Structural Biology 119*:260-272, 1997). While the outer membrane is freely permeable to ionic and non-ionic solutes having molecular weights less than about ten kilodaltons, the inner mitochondrial membrane exhibits selective and regulated permeability for many small molecules, including certain cations, and is impermeable to large (> ~10 kDa) molecules.

Chloroplasts

The chloroplast is an organelle found in plant cells wherein photosynthesis takes place. Photosynthesis, in addition to being an integral part of a plant cell's metabolism, is an important process that impacts many other living organisms as well. The reason for this is twofold: photosynthesis "fixes" atmospheric CO_2 into biologically usable carbohydrate $(CHO)_n$ molecules and also produces O_2 which is required by all aerobic organisms.

Like mitochondria, chloroplasts have a double (outer and inner) membrane, contain their own DNA and have translation factors (ribosomes, tRNAs, *etc.*) that are distinct from those found in the cytoplasm (Sugiura, *Essays Biochem. 30*:49-57, 1995). Electron microscopy demonstrates that, like mitochondria, chloroplasts have a highly organized internal ultrastructure which includes flattened membranous bodies known as lamellae or thykaloid discs. Chloroplasts are, however, typically much larger than mitochondria; in higher plants they are generally cylindrical in shape and range from about 5 to 10 micrometers in length and from 0.5 to 2 micrometers in diameter. Like mitochondria, which are present in greater numbers in certain tissues (*e.g.*, liver) than others, chloroplasts have greater copy numbers in some tissues than others. For example, mature leaves contain many chloroplasts and the total amount of chloroplast DNA in such leaves is about twice that of nuclear DNA (Jope et al., *J. Cell. Biol. 79*:631-636, 1978).

Mitochondrial Electron Transport Chain, $\Delta\Psi$, and Pore Transition

The electron transport chain (ETC) is a mitochondrial activity that drives oxidative phosphorylation to produce metabolic energy in the form of adenosine triphosphate (ATP). Four of the five multisubunit protein complexes (Complexes I, III, IV and V) that mediate ETC activity are localized to the inner mitochondrial membrane; the remaining ETC complex (Complex II) is situated in the mitochondrial matrix. In at least three distinct chemical reactions known to take place within the ETC, protons are moved from the mitochondrial matrix, across the inner membrane, to the intermembrane space. This disequilibrium of charged species creates an electrochemical potential of approximately 220 mV referred to as the "protonmotive force" (PMF). PMF, which is often represented by the notation Δp, corresponds to the sum of the electric potential ($\Delta\Psi m$) and the pH differential (ΔpH) across the inner mitochondrial membrane according to the equation

$$\Delta p = \Delta\Psi m - Z\Delta pH,$$

wherein Z stands for -2.303 RT/F. The value of Z is -59 at 25°C when Δp and $\Delta\Psi m$ are expressed in mV and ΔpH is expressed in pH units (*see, e.g.*, Ernster et al., 1981 *J. Cell Biol. 91*:227s-255s and references cited therein).

Many mitochondrial functions depend in part or entirely on $\Delta\Psi m$. For example, $\Delta\Psi m$ provides the energy for phosphorylation of adenosine diphosphate (ADP) to yield ATP by ETC Complex V, a process that is coupled stoichiometrically with transport of a proton into the matrix. Furthermore, $\Delta\Psi m$ is also the driving force for the influx of cytosolic Ca^{2+} into the mitochondrion. Even fundamental biological processes, such as translation of mRNA molecules to produce polypeptides, appear to be dependent on $\Delta\Psi m$ (Cote et al., *J. Biol. Chem. 265*:7532-7538, 1990).

Under normal metabolic conditions, the inner membrane is impermeable to proton movement from the intermembrane space into the matrix, leaving ETC Complex V as the sole means whereby protons can return to the matrix. When, however, the integrity of the inner mitochondrial membrane is compromised, as occurs during mitochondrial permeability transition (MPT) that accompanies certain diseases associated with altered mitochondrial function, protons are able to bypass the conduit of

Complex V without generating ATP, thereby uncoupling respiration. During MPT, $\Delta\Psi m$ collapses and mitochondrial membranes lose the ability to selectively regulate permeability to solutes both small (*e.g.*, ionic Ca^{2+}, Na^{+}, K^{+}, H^{+}) and large (*e.g.*, proteins).

Mitochondrial Defects, Diseases and Disorders

Mitochondria (or, at least, mitochondrial components) participate in programmed cell death (PCD), also known as apoptosis (Newmeyer et al., *Cell 79*:353-364, 1994; Liu et al., *Cell 86*:147-157, 1996), which is apparently required for normal development of the nervous system and functioning of the immune system. Moreover, some disease states are thought to be associated with either insufficient or excessive levels of apoptosis (*e.g.*, cancer and autoimmune diseases in the first instance, and stroke damage and neurodegeneration in Alzheimer's disease in the latter case). Thus, agents that affect apoptotic events, including those associated with mitochondrial components, might have a variety of palliative, prophylactic and therapeutic uses.

Altered or defective mitochondrial activity, including but not limited to failure at any step of the ETC, may result in the generation of highly reactive free radicals that have the potential of damaging cells and tissues. These free radicals may include reactive oxygen species (ROS) such as superoxide, peroxynitrite and hydroxyl radicals, and potentially other reactive species that may be toxic to cells. For example, oxygen free radical induced lipid peroxidation is a well established pathogenetic mechanism in central nervous system (CNS) injury such as that found in a number of degenerative diseases, and in ischemia (*i.e.*, stroke).

In addition to free radical mediated tissue damage, there are at least two deleterious consequences of exposure to reactive free radicals arising from mitochondrial dysfunction that adversely impact the mitochondria themselves. First, free radical mediated damage may inactivate one or more of the myriad proteins of the ETC. Second, free radical mediated damage may result in catastrophic mitochondrial collapse that has been termed "permeability transition" (PT) or "mitochondrial permeability transition" (MPT). According to generally accepted theories of

mitochondrial function, proper ETC respiratory activity requires maintenance of an electrochemical potential ($\Delta\Psi m$) in the inner mitochondrial membrane by a coupled chemiosmotic mechanism, as described herein. Free radical oxidative activity may dissipate this membrane potential, thereby preventing ATP biosynthesis and halting the production of a vital biochemical energy source. In addition, mitochondrial proteins such as cytochrome c and "apoptosis inducing factor" may leak out of the mitochondria after permeability transition and may induce the genetically programmed cell suicide sequence known as apoptosis or programmed cell death (PCD). Therefore, mere determination of free radical induced damage, such as lipid peroxidation, is not an accurate or early indicator of mitochondrial dysfunction.

Altered mitochondrial function characteristic of the mitochondria associated diseases may also be related to loss of mitochondrial membrane electrochemical potential by mechanisms other than free radical oxidation, and permeability transition may result from direct or indirect effects of mitochondrial genes, gene products or related downstream mediator molecules and/or extramitochondrial genes, gene products or related downstream mediators, or from other known or unknown causes. Loss of mitochondrial potential therefore may be a critical event in the progression of diseases associated with altered mitochondrial function, including degenerative diseases.

Mitochondrial defects, which may include defects related to the discrete mitochondrial genome that resides in mitochondrial DNA and/or to the extramitochondrial genome, which includes nuclear chromosomal DNA and other extramitochondrial DNA, may contribute significantly to the pathogenesis of diseases associated with altered mitochondrial function. For example, alterations in the structural and/or functional properties of mitochondrial components comprising subunits encoded directly or indirectly by mitochondrial and/or extramitochondrial DNA, including alterations deriving from genetic and/or environmental factors or alterations derived from cellular compensatory mechanisms, may play a role in the pathogenesis of any disease associated with altered mitochondrial function. A number of degenerative, hyperproliferative and other types of diseases are thought to be caused

by, or to be associated with, alterations in mitochondrial function. These include, for example, Alzheimer's Disease, Parkinson's Disease, Huntington's disease, diabetes mellitus, and hyperproliferative disorders, such as cancer, tumors and psoriasis. The extensive list of mitochondria associated diseases, *i.e.*, diseases associated with altered mitochondrial function and/or mitochondrial mutations, continues to expand as aberrant mitochondrial or mitonuclear activities are implicated in particular disease processes.

SUMMARY OF THE INVENTION

The invention relates to factors encoded by genes that are differentially expressed in cellular models of particular disease states associated with organelles in cells as compared to control cells, or in cells in response to various compounds or conditions thought to influence organellar function, or in a species-specific manner. In brief, the present invention provides methods for identifying factors that directly or indirectly influence organellar function, or which are over- or under-expressed in organelle-associated diseases and disorders, including but not limited to diseases and disorders associated with mitochondria. Differentially expressed genes and factors in organelle-associated diseases include organellar factors, *i.e.*, macromolecules found within or associated with organelles, and cellular factors that negatively or positively influence, either directly or indirectly, the amount and/or activity of such macromolecules. Organellar factors may be macromolecules found within or associated with organelles, or cellular factors that negatively or positively influence, either directly or indirectly, the amount and/or activity of such macromolecules. Such factors (*e.g.*, gene products) include nucleic acids and proteins that are expressed from genes that are derived from a cell's or an organism's nuclear genome, as well as those expressed from the genomes of organelles such as mitochondria or chloroplasts (*e.g.*, extranuclear genomes). Of particular interest are nucleic acids that are differentially expressed in particular disease states, in response to various compounds or conditions, or in a species-specific fashion.

Thus in one aspect the present invention provides a method for identifying organellar factors encoded by genes that are differentially expressed,

comprising providing at least one cell in a first state, providing at least one cell in a second state, determining the expression of genes in such cells, and identifying genes that are differentially expressed in cells in the first state relative to cells in the second state. The cell(s) in either state may be treated with one or more stressors known or thought to influence organellar function, and the cell(s) in the other state may be control (*e.g.*, untreated) cells.

In another aspect, the invention provides a method for identifying differentially expressed organellar genes in manipulated cells, comprising providing at least one first cell that is not a manipulated cell, providing at least one second cell that is a manipulated cell, determining the expression of genes in the first cell(s) and the second cell(s), and identifying genes that are differentially expressed in the first cell relative to the second cell. Manipulated cells include but are not limited to (a) ρ^0 and cybrid cells, (b) cells that have been genetically engineered to over- or under-express factors known or thought to directly or indirectly influence organellar function, and (c) cells that have been treated with an agent (*e.g.*, an antisense oligonucleotide) that influences organellar function and/or expression of factors associated with organellar function and diseases or disorders. Manipulated cells also includes cells that fall into two or more of the categories (a), (b) and (c); these categories are not mutually exclusive. It is also possible to compare gene expression in a cybrid cell line to ρ^0 cells from which the cybrids were prepared.

In an aspect of the invention related to category (c) of the preceding paragraph (*i.e.*, cells that have been treated with an agent (*e.g.*, an antisense oligonucleotide) that influences organellar function and/or expression of factors associated with organellar function and diseases or disorders), a method is provided for identifying nucleic acids that are differentially expressed during apoptosis, comprising providing at least one first cell that is not in an apoptotic state, providing at least one second cell that is in an apoptotic state, determining the expression of genes in the first cell(s) and the second cell(s), and identifying genes that are differentially expressed in first cell(s) relative to said second cell(s). Apoptosis can be induced by a variety of treatments, as detailed below. In a related aspect of the invention, other agents may

effect, alter (*e.g.*, increase or decrease), influence or otherwise regulate organellar function, including apoptogens at concentrations where apoptosis is not induced. Examples of such compounds include but are not limited to Ruthenium Red, which blocks the action of the mitochondrial calcium uniporter; ionophores such as ionomycin, which increase the intracellular concentration of ions such as Ca^{++}; and uncouplers and/or blockers of the electron transport chain.

It is another aspect of the present invention to provide a method for identifying nucleic acids that are differentially expressed in a species-specific manner, comprising providing at least one cell from a first species, providing at least one cell that is from a second species, determining the expression of genes in the cell(s) from the first species and the cell(s) from the second species, and identifying genes that are differentially expressed in the cell(s) from the first species as compared to the cell(s) from the second species. This aspect of the invention includes methods in which a candidate species-specific agent is tested for its ability to impact the expression of related (homologous) genes in one species and not the other. The cells can additionally or alternatively be treated with an agent that influences organellar function and/or expression of factors associated with organellar function and diseases or disorders, and can be manipulated cells, including but not limited to ρ^0 and cybrid cells.

Accordingly, and as provided herein, in certain aspects the present invention provides a method for identifying a factor encoded by a gene that is differentially expressed, comprising comparing (i) expression of a plurality of genes in at least one first cell that is in a first state to (ii) expression of a plurality of genes in at least one second cell that is in a second state, thereby identifying a gene that is differentially expressed in said first state relative to said second state, and therefrom identifying a factor encoded by a gene that is differentially expressed. In one embodiment the first cell is a manipulated cell and in certain further embodiments the second cell is a manipulated cell. In certain further embodiments the manipulated cell is a cybrid cell, while in certain other embodiments the manipulated cell is a ρ^0 cell. In one embodiment the first cell is a manipulated cell and the second cell is a manipulated cell, and in certain further embodiments at least one of said first and second cells is a

cybrid cell. In certain other further embodiments both of said first and second cells are cybrid cells. In another embodiment at least one of said first and second cells is a ρ^0 cell, and in another embodiment both of said first and second cells are ρ^0 cells.

In certain embodiments the factor is an organellar factor, which in certain other embodiments is protein and in certain other embodiments is a nucleic acid. In certain other embodiments the factor is differentially expressed in an organelle associated disease. In certain other embodiments the factor is differentially expressed in response to treatment with an agent that alters at least one organellar function, which in certain further embodiments is a mitochondrial function and in certain still further embodiments is electron transport chain activity, oxidative phosphorylation, ATP production, intracellular calcium homeostasis, apoptosis, mitochondrial permeability transition or free radical production. In certain other embodiments the factor is differentially expressed in response to treatment with an agent that is a stressor or an apoptogen. In certain other embodiments the factor is differentially expressed in a species specific fashion.

In yet another embodiment, the first state and the second state are different and at least one of the first and second states is a disease state. In one such embodiment, the disease is an organelle associated disease. In another embodiment, the first state and the second state are different and at least one of the first and second states is a response to a stressor, which in certain further embodiments is a molecule and in certain other further embodiments is an environmental factor. In certain embodiments of the present invention, the step of comparing comprises determining mRNA in each of the first and second cells, while in certain other embodiments the step of comparing comprises determining protein in each of the first and second cells. According to certain embodiments, the first and second cells are derived from the same clone, while in certain other embodiments the first and second cells are derived from different species. In another embodiment, the first state and the second state are different and at least one of the first and second states is a metabolic state, a respiratory state, a cell cycle state, a pathologic state, a differentiative state, a maturational state, a genetic state, an apoptotic state, an excitotoxic state or a pharmacological state.

In another embodiment, the invention provides a method of diagnosing a disease comprising contacting a biological sample from an individual suspected of having the disease with at least one factor identified according to the above described method for identifying a factor encoded by a gene that is differentially expressed, comprising comparing (i) expression of a plurality of genes in at least one first cell that is in a first state to (ii) expression of a plurality of genes in at least one second cell that is in a second state, thereby identifying a gene that is differentially expressed in said first state relative to said second state, and therefrom identifying a factor encoded by a gene that is differentially expressed. In one embodiment the factor is a nucleic acid, which in certain further embodiments may have the sequence of SEQ ID NOS:8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22; the reverse complements of SEQ ID NOS:8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22; or an equivalent thereof.

It is another aspect of the present invention to provide a method of diagnosing a disease comprising contacting a biological sample from an individual suspected of having the disease with an antibody that specifically binds a factor identified according to the above described method for identifying a factor encoded by a gene that is differentially expressed, comprising comparing (i) expression of a plurality of genes in at least one first cell that is in a first state to (ii) expression of a plurality of genes in at least one second cell that is in a second state, thereby identifying a gene that is differentially expressed in the first state relative to the second state, and therefrom identifying a factor encoded by a gene that is differentially expressed. In a further embodiment, the factor is a protein.

In another aspect, the invention provides the cybrid cell lines 1685, ATCC 207149 and ATCC 207150.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an electrophoretic gel showing the results (fluorescently labeled PCR products) from a typical differential display (DD) experiment with control

(MixCon) and Alzheimer's (1685) cybrids. The positions of molecular weight markers (b, number of bases) are indicated on the left. Primer pairs (AP, anchored primer; ARP, arbitrary primer) are indicated on the bottom (as an example, "10/1" indicates that the primers AP10 and M13r-ARP1 were used). The numbers on the top indicate the times at which samples were taken ("2w" = 2 weeks; "4w" = 4 weeks; "6w" = 6 weeks). Duplicate reactions were prepared and run in parallel in adjacent lanes. In the figure, certain nucleic acids of interest are boxed and labeled, including MG-NOV2 (a.k.a. 1685 DD-Sequence #4, SEQ ID NO:10), MG-NOV3 (a.k.a. 1685 DD-Sequence #5, SEQ ID NO:11) and YAC 377A1 (a.k.a. 1685 DD-Sequence #2, SEQ ID NO:8).

Figure 2 shows an alignment between 1685 DD-Sequence #1 (SEQ ID NO:7) and human nucleotide sequences derived from the gene encoding 3-hydroxyisobutyryl-coenzyme A hydrolase (GenBank accession No. U66669; SEQ ID NO:64).

Figure 3 shows an alignment between 1685 DD-Sequence #2 (SEQ ID NO:8) and human nucleotide sequences derived from YAC clone 377A1 (GenBank accession No. AF009203; SEQ ID NO:65) and a cDNA encoding an uncharacterized protein designated KIAA0711 (GenBank accession No. AB018254; SEQ ID NO:66).

Figure 4 shows an alignment between 1685 DD-Sequence #3 (SEQ ID NO:9) and human nucleotide sequences derived from BAC clone CIT987-SKA-237H1 (GenBank accession No. AC002287; SEQ ID NO:67).

Figures 5-32 show, respectively, sequences UNK1-UNK28 (SEQ ID NOS: 23-58).

Figure 33 shows an alignment of UNK5 (SEQ ID NO:27), UNK10-5' (SEQ ID NO:32) and UNK10-3' (SEQ ID NO:33) nucleotide sequences.

Figure 34 shows an alignment of UNK19 (SEQ ID NO:45) and UNK18 (SEQ ID NO:44) nucleotide sequences.

Figure 35 shows an alignment of KIAA0138 (encoded by a cDNA that overlaps SEQ ID NO:8) with two human proteins having related amino acid sequences, and a consensus sequence (SEQ ID NO:63) derived therefrom. KIAA0138, uncharacterized protein KIAA0138 (Accession No. ___; SEQ ID NO:62); AK000867,

uncharacterized protein AK000867 (Accession No. ; SEQ ID NO:61); Factor B (SEQ ID NO:60), scaffold attachment factor. Upper case residues in the consensus sequence are conserved in all three proteins; lower case residues indicate variable positions.

Figure 36 shows a sequence (SEQ ID NO:59) that aligns with and overlaps a cDNA (Accession No. X01662) that encodes SOD-1 (superoxide dismutase).

Figure 37 shows the results of various homology searches as explained in the Examples.

Figure 38 shows the results of an EST database sequence alignment search using SEQ ID NO:8.

Figure 39 shows the results of homology searching with an UNK5-derived consensus sequence (SEQ ID NO:8).

FREQUENTLY USED SYMBOLS AND ABBREVIATIONS

$\Delta\psi$, $\Delta\psi m$	mitochondrial membrane potential
ΔpH	pH differential across the inner mitochondrial membrane
AD	Alzheimer's disease
ETC	electron transport chain
MixCon	mixed control
MPT	Mitochondrial Permeability Transition
mtDNA	mitochondrial DNA
NAO	nonyl acridine orange
PD	Parkinson's disease
PMF, Δp	protonmotive force
rho^0, ρ^0	lacking mtDNA

DETAILED DESCRIPTION OF THE INVENTION

In certain embodiments, the present invention is directed to a method of identifying organellar factors encoded by genes that directly or indirectly alter or influence organellar function; and/or that are differentially expressed in particular disease states including organelle associated diseases and disorders including those

described herein; and/or which are differentially expressed in response to treatment with one or more agents thought or known to impact, either directly or indirectly, one or more organellar functions; and/or which are differentially expressed in cells, including manipulated cells, derived from one species relative to cells derived from a second species; and/or that are differentially expressed in response to various stressors or in a species-specific fashion. By "differentially expressed," it is meant that the gene is over- or under-expressed in one cell type, or under one set of conditions, relative to another; accordingly, in certain embodiments the corresponding gene product is present in greater amounts in one cell type, or under one set of conditions, than in another.

Thus, the present invention provides methods for identifying factors, including organellar factors as provided herein, that directly or indirectly influence organellar function, or which are over- or under-expressed in organelle-associated diseases and disorders, including but not limited to diseases and disorders associated with mitochondria. As noted above, organellar factors may be macromolecules found within or associated with organelles, or cellular factors that negatively or positively influence, either directly or indirectly, the amount and/or activity of such macromolecules. Such factors (*e.g.*, gene products) include nucleic acids and proteins that are expressed from genes that are derived from a cell's or an organism's nuclear genome, as well as those expressed from the genomes of organelles such as mitochondria or chloroplasts. Of particular interest are nucleic acids that are differentially expressed in particular disease states, in response to various compounds or conditions, or in a species-specific fashion. Therefore, differentially expressed genes and factors in organelle associated diseases as provided herein include organellar factors.

In one aspect of the present invention there is provided a method for identifying factors, which in certain embodiments are organellar factors, encoded by genes that are differentially expressed, comprising providing at least one cell in a first state, providing at least one cell in a second state, determining the expression of genes in such cells, and identifying genes that are differentially expressed in cells in the first state relative to cells in the second state. The cell(s) in either state may be treated with

one or more stressors known or thought to influence organellar function, and the cell(s) in the other state may be control (untreated) cells. The state of a cell as provided herein includes the biological or physiological status or condition of the cell, for example, the metabolic, respiratory, cell cycle (*e.g.*, mitotic), pathologic, differentiative, maturational, genetic (*e.g.*, ploidy, homoplasmic, heteroplasmic, nuclear genetic, extranuclear genetic, etc.), apoptotic, electrochemical, adhesive, activational, excitotoxic or pharmacological status or the like. Preferably, the first state and the second state are different regarding a particular disease state, which may in certain embodiments be an organelle associated disease state. In certain other embodiments the first state and the second state may differ with respect to the presence and/or effects of a stressor. The stressor can be any stressor, but is preferably a molecule or an environmental factor. The determining step preferably includes determining the mRNA or protein in the cell(s) in the first state or the cell(s) in the second state, preferably both. Preferably, the cell(s) in the first state and the cell(s) in the second state are clonally derived and/or are derived from the same organism. The identifying step preferably includes comparing the mRNA or protein in the cell(s) in the first state and the cell(s) in the second state. Accordingly, in certain preferred embodiments of the invention there is provided a method of identifying a differentially expressed factor that is an organellar factor as provided herein.

In another aspect the invention provides a method for identifying differentially expressed genes, for example organellar genes, in manipulated cells, comprising providing at least one first cell that is not a manipulated cell, providing at least one second cell that is a manipulated cell, determining the expression of genes in the first cell(s) and the second cell(s), and identifying genes that are differentially expressed in the first cell relative to the second cell. Manipulated cells include but are not limited to (a) ρ^0 and cybrid cells, (b) cells that have been genetically engineered to over- or under-express factors known or thought to directly or indirectly influence organellar function, and (c) cells that have been treated with an agent (*e.g.*, an antisense oligonucleotide) that influences organellar function and/or expression of factors associated with organellar function and diseases or disorders. Manipulated cells also

includes cells that fall into two or more of these categories (a), (b) and (c); these categories are not mutually exclusive.

In an aspect of the invention related to category (c) of the preceding paragraph (cells that have been treated with an agent (*e.g.*, an antisense oligonucleotide) that influences organellar function and/or expression of factors associated with organellar function and diseases or disorders), a method is provided for identifying nucleic acids that are differentially expressed during apoptosis, comprising providing at least one first cell that is not in an apoptotic state, providing at least one second cell that is in an apoptotic state, determining the expression of genes in the first cell(s) and the second cell(s), and identifying genes that are differentially expressed in first cell(s) relative to said second cell(s). Apoptosis can be induced by a variety of treatments, as detailed below. In a related aspect of the invention, other agents that impact organellar function, including apoptogens at concentrations where apoptosis is not induced. Examples of such compounds include but are not limited to Ruthenium Red, which blocks the action of the mitochondrial calcium uniporter; ionophores such as ionomycin, which increase the intracellular concentration of ions such as Ca^{++}; and uncouplers and blockers of the electron transport chain.

The invention also provides, in another aspect, a method for identifying nucleic acids that are differentially expressed in a species-specific manner, comprising providing at least one cell from a first species, providing at least one cell that is from a second species, determining the expression of genes in the cell(s) from the first species and the cell(s) from the second species, and identifying genes that are differentially expressed in the cell(s) from the first species as compared to the cell(s) from the second species. This aspect of the invention includes methods in which a candidate species-specific agent is tested for its ability to impact the expression of related (homologous) genes in one species and not the other. The cells can additionally or alternatively be treated with an agent that influences organellar function and/or expression of factors associated with organellar function and diseases or disorders, and can be manipulated cells, including but not limited to ρ^0 and cybrid cells.

Definitions and General Methods

The following definitions and general methods are applicable to the present invention. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Generally, the nomenclature used herein and the laboratory procedures in cell culture, chemistry, microbiology, molecular biology, cell science and cell culture described below are well known and commonly employed in the art. Conventional methods are used for these procedures, such as those provided in the art and various general references (Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd edition, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989)). Where a term is provided in the singular, the inventors also contemplate the plural of that term. The nomenclature used herein and the laboratory procedures described below are those well known and commonly employed in the art.

Detecting Differentially Expressed Nucleic Acids

A variety of methods and means for detecting differentially expressed nucleic acids may be used in the methods of the invention. Differential Display (DD) and Quantitative Real-Time Polymerase Chain Reaction (Q-RTPCR) are described in detail in the Examples of the disclosure; some other methods and means include, without limitation, the following methodologies. It should be noted that, regardless of which method is used to initially identify candidate differentially expressed genes, a second independent method is preferably used to verify the results obtained from the first method.

Subtractive Hybridization: In a typical procedure for applying the technique of subtraction hybridization (Hedrick et al., *Nature 308*:149-153, 1984) to investigate differences in the active genes of a certain sample of test or target cells, *e.g.*, from tumor tissues, as compared with the active genes of a sample of reference cells, *e.g.*, cells from corresponding normal tissue, total cell mRNA is extracted (using any preferred method) from both samples of cells. The mRNA in the extract from the test or target cells is then used in a conventional manner to synthesize corresponding single

stranded cDNA using an appropriate primer and a reverse transcriptase in the presence of the necessary deoxynucleoside triphosphates, and the template mRNA is subsequently degraded by alkaline hydrolysis or RNase H to leave only the single stranded cDNA. The single stranded cDNA thus derived from the mRNA expressed by the test or target cells is then mixed under hybridizing conditions with an excess quantity of the mRNA extract from the reference (normal) cells; this mRNA is generally termed the subtraction hybridization "driver" since it is this mRNA or other single stranded nucleic acid present in excess which "drives" the subtraction process. As a result, cDNA strands having common complementary sequences anneal with the mRNA strands to form mRNA/cDNA duplexes and are thus subtracted from the single stranded species present. The only single stranded DNA remaining is then the unique cDNA that is derived specifically from the mRNA produced by genes which are expressed solely by the test or target cells.

From this point onwards, to complete the subtraction process and use the single stranded unique cDNA, for example for producing labeled probes that may perhaps then be used for detecting or identifying corresponding cloned copies in a cDNA clone colony (labeling of such probes is frequently introduced by using labeled deoxynucleoside triphosphates in synthesis of the cDNA), it is generally necessary to physically to separate out the common mRNA/cDNA duplexes, using for example hydroxyapatite (HAP) or (strept)avidin-biotin in a chromatographic separation method. Finally, one or more repeat rounds of the subtraction hybridization may be carried out to improve the extent of recovery of the desired product, although other means may be employed (see, *e.g.*, U.S. Patent No. 5,589,339).

High Density Arrays: Multiple sample nucleic acid hybridization analysis can be carried out on micro-formatted multiplex or matrix devices (*e.g.*, DNA or RNA chips, filters and microarrays) (see, *e.g.*, Bains, *Bio/Technology 10*:757-758, 1992). These hybridization formats are micro-scale versions of the conventional "dot blot" and "sandwich" hybridization systems. In these methods, specific DNA sequences are typically attached to, or synthesized on, very small specific areas of a solid support, allowing large numbers of different DNA sequences to be placed in a

small area. The high density arrays comprise target elements, *i.e.*, target nucleic acid molecules bound to a solid support. The nucleic acids for both the target elements and the probes may be, for example, RNA, DNA, or cDNA. In one type of array, target elements comprising nucleic acid elements that are short synthetic oligonucleotides derived from mRNA, cDNA or EST sequences are used to carry out serial analysis of gene expression (SAGE; U.S. Patent No. 5,866,330).

In methods for comparing two nucleic acid collections, nucleic acid molecules in the test and control collections (which may be, *e.g.*, mRNA preparations from a diseased and undiseased human) are detectably labeled. The first and second labeled probes thus formed are each contacted to an identical high density array comprising a plurality of target elements under conditions such that nucleic acid hybridization to the target elements can occur.

After contacting the probes to the target elements the amount of binding to each target element in each of the two arrays is measured, and the binding ratio (*i.e.*, amount bound in the disease sample / amount bound in the control sample) is determined for each target element. A binding ratio >1 indicates that nucleic acids hybridizing to the particular target element are "up-regulated" in the nucleic acid collection prepared from the diseased patient relative to the nucleic acid prepared from the control individual, whereas a binding ratio <1 indicates that nucleic acids hybridizing to the particular target element are "down-regulated" in the diseased patient.

High density cDNA arrays that may be used in the invention include but are not limited to GeneChip™ arrays comprising synthetic oligonucleotides (Affymetrix, Inc., Santa Clara, CA); GeneFilters™ yeast or human cDNA arrays (Research Genetics, Huntsville, AL); ATLAS™ cDNA arrays (Clontech); and GEM™ and Gene Display Arrays (GDA) cDNA arrays (Genome Systems, Inc., St. Louis, MO). Furthermore, one method for building a microarrayer (a machine that produces microarrays) is available on-line at http://cmgm.stanford.edu/pbrown/mguide/index.html.

One type of high density arrays uses electronic hybridization, *i.e.*, a method that directs sample DNA molecules to, and concentrates them at, test sites on a

microchip that can be electronically activated by a positive charge. Because DNA molecules in solution have strong negative charges, they are attracted to activated sites. The electronic hybridization of sample DNA molecules at each test site promotes rapid hybridization of the sample DNAs with the nucleic acids of the target elements. Materials for electronic hybridization are available from Nanogen (San Diego, CA) and the method is described in U.S. Patent No. 5,849,486.

Manipulated Cells

In the present disclosure, the term "manipulated cells" refers to cells that have been altered by human manipulation, such manipulation often (but not necessarily) occurring *in vitro*. Manipulated cells include, but are not limited to, cybrids, rho^0 cells, and cells that have been genetically manipulated in one fashion or another.

It is known in the art to prepare cellular hybrids (cybrids) having a cytoplasmic component, which typically includes organelles such as mitochondria or chloroplasts, from one cell line and a nuclear component from another cell line. Experiments with such cybrids have demonstrated that cellular defects associated with diseased cells are transferred with cytoplasmic elements (mitochondria) from diseased cells to cybrids. Human diseases that have been demonstrated to have a cytoplasmic component in this manner include Alzheimer's disease and Parkinson's disease (Swerdlow et al., *Neurology 49*:918-925, 1997; Davis et al., *Proc. Natl. Acad. Sci. (USA) 94*:4526-4531, 1997; Swerdlow et al., *Annals of Neurology 40*:663-671, 1996).

In some embodiments of the invention, differentially expressed factors are defined as factors that have a pattern of expression in "disease cybrids" (*i.e.*, cybrids having a cytoplasmic component derived from one or more individuals known to have or suspected of having a disease of interest) that is different from the pattern of expression observed in "control cybrids" (*i.e.*, cybrids having a cytoplasmic component derived from one or more individuals not having the disease of interest). One advantage of using cybrid cells for experiments designed to identify the differential expression of factors involved in organellar functions is that disease and control cybrids share commonly-derived nuclear components. Differences in expression patterns

between various cybrids are thus more likely to be due solely to differences in cytoplasmic components and not to differences in the nuclear genome.

With regard to animal cells, methods for preparing cellular hybrids (cybrids) comprising the nucleus of one cell type and organelles (mitochondria) from another cell type have been described (see published PCT application No. PCT/US95/04063, U.S. patent application Serial No. 09/069,489, and U.S. Patent No. 5,840,493, all of which are hereby incorporated by reference). In a particular embodiment of the invention, differentiable cybrid cell lines are used to carry out differential expression experiments (see U.S. patent application Serial No. 08/397,808, now U.S. Patent No. 5,888,498, hereby incorporated by reference).

Cybrid plant cells have also been described (see, for example, U.S. Patents 4,751,347 and 5,360,725, hereby incorporated by reference). In one embodiment of the invention, plant cybrids are used in differential expression experiments to identify factors related to functions of organelles (mitochondria and/or chloroplasts) in plants. In another embodiment of the invention, factors that are differentially expressed in plant cells comprising genetically engineered chloroplasts (U.S. Patent No. 5,693,507, hereby incorporated by reference) relative to plant cells having wildtype chloroplasts are identified. Factors identified by these embodiments of the invention are useful for agricultural applications such as, *e.g.*, increasing the lifespan, productive capacity, and/or insecticide or herbicide resistance of crops.

In general, cybrids are prepared by first preparing cells that lack mitochondria; such cells are known as rho^0 cells. In a further embodiment of the invention, a differentially expressed factor is defined as a factor that has a pattern of expression in rho^0 cells that is different from the pattern of expression observed in the parent rho^+ (mitochondria-containing) cells. Methods for preparing rho^0 cells for a variety of cell types (animal, fungal, etc.) are known in the art. By way of example and not limitation, yeast rho^0 cells can be prepared by ethanol treatment (Ibeas and Jimenez, Appl. Environ. Microbiol. 63:7-12, 1997), and a variety of mammalian rho^0 cells can be prepared by treatment with ditercalinium (Inoue et al., *Biochem. Biophys. Res. Commun. 239*:257-260, 1997), ethidium bromide (King and Attardi, *Science 246*:500-

503, 1989; Cavalli et al., *Cell Growth Differ.* 8:1189-1198, 1997; Miller et al., *J. Neurochem.* 67:1897-1907, 1996) and various antiviral agents (U.S. patent application Serial No. 09/069,489).

Methods and compositions for the genetic manipulation of the mitochondrial genome of the yeast species *Saccharomyces cerevisiae* have been described in the art (Steele et al., *Proc. Natl. Acad. Sci. U.S.A.* 93:5253-5257, 1996). Another embodiment of the invention is drawn to the identification and isolation of factors that are differentially expressed in yeast cells having genetically engineered mitochondrial genomes relative to yeast cells having wildtype mitochondrial genomes.

Manipulated cells includes the preceding cell types in which an organellar genome has been altered by human manipulation; additionally or alternatively, such cells may comprise alterations in their nuclear genomes (such as, *e.g.*, point mutations or "knock-outs" in chromosomal nucleic acid sequences) or in non-organellar, extrachromosomal elements (such as, *e.g.*, plasmids, viruses, and the like). In the latter instance, genetic elements from a species different from that to which the host cell belongs may be introduced into the manipulated cell on the extrachromasomal element, in which case differentially expressed factors are those factors having an altered pattern of expression in response to the exogenic element(s).

Nucleic Acids and Nucleotide Sequences

A "nucleic acid of interest" is defined herein as a nucleic acid that is differentially expressed in a particular disease state, under particular conditions, in manipulated cells, or in a species-specific manner, as described above. Once a nucleic of interest has been identified, it can be used to generate other useful nucleic acids having related sequences, including without limitation deoxyribonucleic acids (DNA). In a preferred embodiment, an RNA of interest is used to generate a cDNA molecule that can be used to detect nucleic acids having the sequence of interest, or to produce a polypeptide encoded by the sequence of the RNA of interest.

For example, it is known in the art to isolate mRNAs of interest and have them reverse-transcribed. Reverse transcription is a process by which a reverse

complementary DNA (cDNA) is produced from an RNA molecule which acts as a template. The RNA portion of the resultant (RNA:DNA) hybrid may then be displaced or enzymatically degraded, after which the single-stranded DNA (ssDNA) is used as a template for one or more rounds of DNA polymerization, the product of which is a double-stranded DNA (dsDNA) molecule. The dsDNA molecule includes the sequence of the RNA of interest (except that uridine residues in the RNA are replaced by thymine residues in the DNA). The nucleotide sequence of the dsDNA is then determined and analyzed; additionally or alternatively, the dsDNA is cloned, *i.e.*, incorporated into a vector DNA that is capable of replication in an appropriate host cell. If the dsDNA molecule includes a sequence that encodes a polypeptide, a preferred vector is an expression vector.

A DNA molecule prepared according to the methods of the invention can be a full-length cDNA, *i.e.*, one comprising a nucleotide sequence that encodes an entire protein. At a minimum, a full-length cDNA will encompass a "start" (translation initiating) codon, a "stop" (translation terminating) codon, and all the polypeptide-encoding sequences in-between. Such an assemblage of elements is known in the art as an open reading frame (ORF).

Alternatively, a DNA molecule prepared according to the methods of the invention can be an Expressed Sequence Tag (EST), *i.e.*, one which does not comprise a complete ORF but which does comprise a nucleotide sequence that is a portion of an ORF or of an mRNA comprising an ORF. An EST is useful in of itself as, *e.g.*, a probe in methods for detecting a mRNA of interest. Because a full-length cDNA is required for, *e.g.*, recombinant DNA expression of a protein encoded by a mRNA interest, it may also be desirable to use an EST as a tool to isolate a full-length cDNA according to a variety of methods. For example, a nucleic acid comprising an EST sequence of interest can be labeled and used to probe preparations of cellular DNA or RNA for hybridizing sequences, and such hybridizing sequences can be isolated, amplified and cloned according to known methods. As another example, the sequence of an EST can be used to prepare primers for inverse PCR, a process by which sequences flanking an EST of interest can be determined (see, *e.g.*, Benkel and Fong, *Genet. Anal. 13*:123-

127, 1996; Silverman, *Methods Mol. Biol. 54*:145-155, 1996; Pang and Knecht, *BioTechniques 22*:1046-1048, 1997; Huang, *Methods Mol. Biol. 69*:89-96, 1997; Huang, *Methods Mol. Biol. 67*:287-294, 1997; and Offringa and van der Lee, *Methods Mol. Biol. 49*:181-195, 1996; all of which are hereby incorporated by reference).

In methods of cloning full-length cDNAs from ESTs, and as a useful method in its own right, it is desirable to screen mRNA or cDNA libraries prepared from various cells and tissues in order to identify cells and tissues that express relatively high levels of a nucleic acid of interest. For example, a nucleic acid of interest initially identified in a first disease state (*e.g.*, Alzheimer's disease) can be used to probe cells from patients suffering from a second disease state (*e.g.*, Parkinson's disease, MELAS, MERFF, diabetes, cancer, arthritis, *etc.*) in order to determine if the nucleic acid of interest is differentially expressed in such second disease states. If a nucleic acid of interest is differentially expressed in a concordant manner in one or more second disease states, then applications developed from a first disease state (*e.g.*, diagnostic, prognostic, pharmacogenomic, compound screening methods and therapeutic compounds and compositions) may be applied to such second disease states.

As another example, a nucleic acid of interest can be used to examine tissue- or temporal-specific patterns of expression of a nucleic acid of interest in a variety of methods known in the art. The nucleic acid of interest can be detectably labeled and used to probe (i) an immobilized collection of mRNA molecules (*e.g.*, RNA Master Blots™ or Multiple Tissue Northern, MTN™, Blots from Clontech) or (ii) a cDNA library (prepared according to methods known in the art or available from, *e.g.*, Clontech or from depositories such as the American Type Culture Collection, ATCC, Manassas, VA). Alternatively or additionally, a sequence of interest can be used to design specific PCR primers that can be used in amplification reactions in 96-well plates wherein each well comprises first strand cDNAs from a particular tissue (such as, *e.g.*, the Rapid-Scan™ gene expression panel from OriGene Technologies, Inc., Rockville, MD); in this embodiment, automated, semi-automated or robotic means may be used to carry out such assays.

Regardless of the method used, the RNA or cDNA that is examined may be from a variety of species, including without limitation mammals such as porcine species, rabbits, bovine species, rodent species (rats and mice) and primates including humans; avian species such as chicken or turkey; fish such as *Fugu* species; and simple or complex plants such as *Arabidopsis* species, *Zea mays*, potatoes, soybeans, rice, wheat and the like. Mammalian tissues that may be examined include but are not limited to brain (including, by way of example but not limitation, whole brain and subsections thereof, *e.g.*, amygdala, caudate nucleus, cerebellum, cerebral cortex, frontal lobe, hippocampus, medulla oblongata, occipital lobe, putamen, substantia nigra, temporal lobe, thalamus, acumens, subthalamic nucleus), heart, kidney, spleen, liver, colon, lung, small intestine, stomach, skeletal muscle, smooth muscle, testis, uterus, bladder, lymph nodes, spinal cord, trachea, bone marrow, placenta, salivary glands, thyroid glands, thymus, adrenal glands, pancreas, ovary, uterus, prostate, skin, bone marrow, fetal brain and fetal liver.

Cell types that can be probed in this manner include, without limitation, plant and animal cybrids and rho^0 cells; cells from organisms such as, for example, any unicellular organism, multicellular organism, yeast, fungi, protozoa, parasites, helminths, invertebrates or vertebrates or other organisms as they are known in the art or later identified having mitochondria, chloroplasts or other organelles, such as, for example, *Caenorhabditis, Neurospora, Spodoptera, Trichopolusia, Phycomycetes, Ascomycetes, Basidiomycetes, Deuteromycetes, Mycosporum, Trichophyton, Nannizia, Arthroderma, Crytptococcus, Coccidioides, Histoplasma, Blastomyces, Candidia, Cryptococcus, Histoplasma, Saccharomyces, Trichosporon, Coccidioides, Aspergillus, Phycomycetes, Sporothrix, Microsporum, Penicillium, Cladosporium, Alternaria, Geotrichum, Fusarium, Acremonium, Scopulariopsis, Beauveria, Trichophyton, Eidermophyton, Fusarium, Trichosporon, Phialophora, Trichophyton, Epidermophyton, Paracoccidioides, Sporothrix, Pityriasis, Entamoeba, Balantidium, Naegleria, Acanthamoeba, Giardia, Isospora, Cryptosporidium, Enterocytozoon, Trichomonas, Plasmodium, Babesia, Trypanosoma, Leishmania, Toxoplasma, Caenorhabditis elegans, Neurospora crassa, Saccharomyces cerevisae, Spodoptera*

frugiperda, Trichopolusia ni, Xenopus laevis any species or related species thereof (Davis et al., Microbiology, Harper and Row, Philadelphia (1980); O'Learly, Practical Handbook of Microbiology, CRC Press, Boca Raton, (1989); Baron et al., Diagnostic Microbiology, The C.V. Mosby Company, St. Louis (1990) and Robbins, Pathologic Basis of Disease, W.B. Saunders Co, Philadelphia (1994); culturable insect cell lines such as Sf9 and Sf21; cells isolated from mammals such as peripheral blood leukocytes (PBLs), chondriocytes, and the like; culturable mammalian cell lines such as differentiable and differentiated cell lines, cultured neuronal cell lines such as SH-SY5Y or NT2 cells, cultured tumor or cancer cell lines such as Hela cells, cells isolated from or primary cell cultures derived from human patient suffering from diseases and disorders known or suspected of having a mitochondrial component (as defined herein) and manipulated cells (as defined herein) derived from any of the preceding. Such cells are obtained with informed consent from patients suffering from such diseases or disorders, or, in the case of culturable cell lines, are available from a variety of commercial sources or from depositories such as the ATCC.

In order to identify tissues or cells from which a cDNA corresponding to an EST of interest can optimally be prepared, mRNA or cDNA libraries or arrays derived from the organism from which the EST of interest was isolated are probed. Tissues or cells having a high level of expression of the nucleic acid of interest are preferably used as sources for full-length nucleic acids, *i.e.*, nucleic acids containing all the genetic information required to express a complete gene product of interest. The full-length nucleic acids are used, *e.g.*, to express the gene product (*i.e.*, RNA or protein) of interest or to prepare manipulated cells or transgenic animals in which the level of expression or activity, or tissue- or temporal-specific patterns of expression, of the gene product of interest is altered relative to the wildtype condition.

Another utility of ESTs and full-length cDNAs is to search *in silico* for corresponding protein sequences, in order to identify proteins of interest encoded thereby and to prepare antibodies thereto. For example, the nucleotide sequence of an EST or cDNA of interest is translated *in silico* in all six potential reading frames (three reading frames on each strand of a dsDNA), and the resulting amino acid sequences are

used as probes to search protein databases for a match to a portion of a protein having a known amino acid sequence. In the case of mitochondrial proteins, it is desirable to perform such *in silico* translations using both the "universal" genetic code and the somewhat different genetic code utilized in mitochondria (Table 1), as different amino acid sequences will result in each case.

TABLE 1: Differences Between the "Universal" and Mitochondrial Genetic Codes

Codon	"Universal" Genetic Code	Yeast Mitochondrial Genetic Code	Mammalian Mitochondrial Genetic Code
AGA	Arg	Arg	(stop)
AGG	Arg	Arg	(stop)
AUA	Ile	Met	Met
CUA	Leu	Thr	Leu
UGA	(stop)	Trp	Trp

Nucleic acids having or comprising a sequence of interest can be prepared by a variety of methods known in the art. For example, such nucleic acids can be made using molecular biology or synthetic techniques (Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press (1989)). Many equivalent bases in nucleotide sequences are known in the art. For example, thymine (T) residues in DNA are transcribed into uracil (U) residues in RNA molecules but, because both T and U specifically pair with adenine (A) residues, these changes do not impact hybridization specificity. Nucleic acids comprising such equivalent substitutions are within the scope of the disclosure.

As another example, such nucleic acids can be oligonucleotides, including oligodeoxyribonucleotides and oligodeoxynucleotides synthesized *in vitro* by, for example, the phosphotriester, phosphoramidite or H-phosphanate methodologies (see, respectively, Christodoulou, "Oligonucleotide Synthesis: Phosphotriester Approach," Chapter 2 In: *Protocols for Oligonucleotides and Analogs: Synthesis and Properties*, Agrawal, ed., Methods in Molecular Biology Vol. 20, Humana Press, Totowa, NJ (1993); Beaucage, "Oligodeoxyribonucleotide Synthesis: Phosphoramidite

Approach," Chapter 3, *Id.*; and Froehler, "Oligodeoxynucleotide Synthesis: H-phosphonate Approach," Chapter 4, *Id.*, all of which are hereby incorporated by reference).

The length of a nucleic acid according to the present invention can be chosen by one skilled in the art depending on the particular purpose for which the nucleic acid is intended. For PCR primers and antisense oligonucleotides, the length of the nucleic acid is preferably from about 10 to about 50 base nucleotides (nt), more preferably from about 12 to about 30 nt, and most preferably from about 15 to about 25 nt. For probes, the length of the nucleic acid is preferable from about 10 to about 5,000 nt, more preferably from about 15 to about 500 nt, and most preferably from about 20 to about 100 nt.

Appropriate chemical modifications to nucleic acids of the invention are also readily chosen by one skilled in the art. Such modifications may include, for example, means by which the nucleic acid is detectably labeled for use as a probe. Typical detectable labels include radioactive moieties and reporter groups such as, *e.g.*, enzymes and fluorescent or luminescent moieties. Other chemical modifications appropriate for particular uses, such as antisense applications, as explained herein.

Detectably labeled nucleic acids are preferred for diagnostic, prognostic and pharmacogenomic methods of the invention. Whether labeled or unlabeled, nucleic acids of the invention can be provided in kit form, *e.g.*, in a single or separate container, along with other reagents, buffers, enzymes or materials to be used in practicing at least one method of the invention. The kit can be provided in a container that can optionally include instructions or software for performing a method of the invention. Such instructions or software can be provided in any language or human- or machine-readable format.

Machine Readable Formats and Data Processing Systems

The invention is drawn not only to nucleic acids having or comprising a nucleotide sequence of interest or proteins or polypeptides having or comprising an amino acid sequence of interest, but also to such sequences *per se* when provided in a

format, such as data, such as data in a patentable format. Thus, for example, the present invention encompasses a format such as a machine-readable format comprising data such as one or more nucleotide sequences or amino acid sequences of interest as determined or isolated according to the present invention. The format can also include one or more nucleotide sequences or amino acid sequences obtained from other sources, such as databases of such sequences.

For example, the invention includes data in any format, preferably provided in a medium of expression such as printed medium, perforated medium, magnetic medium, holographs, plastics, polymers or copolymers such as cycoolifin polymers. Such data can be provided on or in the medium of expression as an independent article of manufacture, such as a disk, tape or memory chip, or be provided as part of a machine, such as a computer, that is either processing or not processing the data, such as part of memory or part of a program. The data can also be provided as at least a part of a database. Such database can be provided in any format, leaving the choice or selection of the particular format, language, code, selection of data, form of data or arrangement of data to the skilled artisan. Such data is useful, for example, for comparing sequences obtained by the present invention with known sequences to identify novel sequences.

One aspect of the invention is a data processing system for storing and comparing at least a portion of data provided by the present invention. The data processing system is useful for a variety of purposes, for example, for storing, sorting or arranging such data in, for example, database format, and for comparing such data to other data, including data of the present invention or from other sources (for example, GENBANK or SWISPROT). Such a data processing system can include two or more of the following elements in any combination:

I. A computer processing system, such as a central processing unit (CPU). A storage medium or means for storing data, including at least a portion of the data of the present invention or at least a portion of compared data, such as a medium of expression, such as a magnetic medium or polymeric medium;

II. A processing program or means for sorting or arranging data, including at least a portion of the data of the present invention, preferably in a database format, such as a database program or an appropriate portion thereof such as they are known in the art (for example EXCEL or QUATROPRO);

III. A processing program or means for comparing data, including at least a portion of the data of the present invention, which can result in compared data, such as nucleic acid or amino acid comparing programs or an appropriate portion thereof, such as they are known in the art [for example BLAST (http://ncbi.nlm.nih.gov/BLAST (March 7, 1999) and Altschul et al., Nucleic Acids Res. 25:3389-3402 (1997)), ALLIGN, GAP, BESTFIT, FASTA and TFASTA (Wisconsin Genetics Software Page Release 7.0, Genetics Computer Groups, Madison, WI)];

IV. A processing program or means for analyzing at least a portion of the data of the present invention, compared data, or a portion thereof, particularly statistical analysis, such as programs for analyzing nucleic acid or amino acid sequences or statistical analysis programs or an appropriate portion thereof as they are known in the art [for example SAS, BLAST (http://ncbi.nlm.nih.gov/BLAST (March 7, 1999) and Altschul et al., Nucleic Acids Res. 25:3389-3402 (1997)), ALLIGN, GAP, BESTFIT, FASTA and TFASTA (Wisconsin Genetics Software Page Release 7.0, Genetics Computer Groups, Madison, WI)];

V. A formatting processing program or means that can format an output from the data processing system, such as data of the present invention or a portion thereof or compared data or a portion thereof, such as database management programs or word-processing programs, or appropriate portions thereof as they are known in the art; or

VI. An output program or means to output data, such as data of the present invention or a portion thereof or compared data or a portion thereof in a format useful to an end user, such as a human or another data processing system, such as database management programs or word-processing programs or appropriate portions thereof as they are known in the art. Such formats useful to an end user can be any

appropriate format in any appropriate form, such as in an appropriate language or code in an appropriate medium of expression.

See, generally, United States Patent No. 5,138,695 to Means et al., issued August 11, 1992; United States Patent No. 5,325,298 to Gallant, issued June 28, 1994; United States Patent No. 5,398,300 to Levey, issued March 14, 1995; United States Patent No. 5,471,627 to Means et al., issued November 28, 1995; United States Patent No. 5,619,709 to Caid et al., issued April 8, 1997; United States Patent No. 5,745,654 to Titan, issued April 28, 1998; United States Patent No. 5,687,306 to Blank, issued November 11, 1997; United States Patent No. 5,577,179 to Blank, issued November 19, 1996; United States Patent No. 5,469,536 to Blank, issued November 21, 1995 and United States Patent No. 5,345,313 to Blank, issued September 6, 1994.

When the nucleotide sequence of interest encodes a protein, the invention is further drawn to the corresponding polypeptide sequences provided in such formats. Such formats are useful in, *e.g.*, diagnostic, prognostic or pharmacogenomic assays useful in the methods of the invention, or in methods for searching *in silico* for homologs of the sequences of interest.

Expression Systems

In order to produce a gene product of interest in sufficient quantities for further embodiments of the invention, the nucleotide sequence of interest or its functional equivalent, is inserted into an appropriate "expression vector," *i.e.*, a genetic element, often capable of autonomous replication, which contains the necessary elements for the transcription and, in instances where the gene product is a protein, translation of the inserted nucleotide sequence. A genetic element that comprises an expression vector and a nucleic acid of interest in an arrangement appropriate for expression of a gene product of interest is referred to herein as an "expression construct."

Methods which are well known to those skilled in the art can be used to prepare expression constructs containing a nucleotide sequence of interest and appropriate transcriptional and translational controls. These methods include *in vitro*

recombinant DNA techniques, synthetic techniques and *in vivo* recombination or genetic recombination. Such techniques are known in the art (see, *e.g.*, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Press, Plainview N.Y., 1989; Ausubel et al., eds., *Short Protocols in Molecular Biology*, Second Edition, John Wiley & Sons, New York N.Y., 1992).

A variety of expression vector/host systems may be utilized to contain and express a nucleotide sequence of interest. These include but are not limited to microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (*e.g.*, baculovirus); plant cell systems transfected with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmid); or animal cell systems.

The "control elements" or "regulatory sequences" of these systems, which may vary in their strength and specificities, are those non-translated regions of the vector, enhancers, promoters, and 5' and 3' untranslated regions, which interact with host cellular proteins to carry out transcription and, where the gene product of interest is a protein, translation. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid *lacZ* promoter of the Bluescript™ phagemid (Stratagene, La Jolla, CA.) or pSport1 (Life Technologies, Inc., Rockville, MD) and p*trp-lac* hybrids and the like may be used. In insect cells, the baculovirus polyhedrin promoter may be used in insect cells. Promoters and/or enhancers derived from the genomes of plant cells (*e.g.*, heat shock, RUBISCO; and storage protein genes) or from plant viruses (*e.g.*, viral promoters or leader sequences) may be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are appropriate. If it is necessary to generate a cell line that contains multiple copies of the nucleotide sequence of interest, vectors based on SV40 or EBV may be used with an appropriate selectable marker.

In bacterial systems, a number of expression vectors may be selected depending upon the use intended for expressed gene product of interest. For example, when large quantities of a protein of interest are needed for the induction of antibodies, vectors which direct high level expression of the protein of interest, or fusion proteins derived therefrom that are more readily assayed and/or purified, may be desirable.

Such vectors include, but are not limited to, *Escherichia coli* cloning and expression vectors such as pET (Stratagene, La Jolla, CA), pRSET (Invitrogen, Carlsbad, CA) or pGEMEX™ (Promega, Madison, WI) vectors, in which the sequence encoding a protein of interest is ligated downstream from a bacteriophage T7 promoter and ribosome binding site so that, when the expression construct is transformed into *E. coli* expressing the T7 RNA polymerase, large levels of the polypeptide of interest are produced; pGEM™ vectors (Promega), in which inserts into sequences encoding the *lacZ* α-peptide may be detected using colorimetric screening; and the like. For polypeptides that are relatively insoluble, it may be desirable to produce thioredoxin fusion proteins using, for example, pBAD/Thio-TOPO vectors (Invitrogen).

Plasmids such as pGEX vectors (Amersham Pharmacia Biotech, Piscataway, NJ) may be used to express polypeptides of interest as fusion proteins. Such vectors comprise a promoter operably linked to a glutathione S-transferase (GST) gene from *Schistosoma japonicum*. (Smith et al., 1988, *Gene 67*:31-40), the coding sequence of which has been modified to comprise a thrombin cleavage site-encoding nucleotide sequence immediately 5' from a multiple cloning site. GST fusion proteins can be detected by Western blots with anti-GST or by using a colorimetric assay; the latter assay utilizes glutathione and 1-chloro-2-4-dinitrobenzene (CDNB) as substrates for GST and yields a yellow product detectable at 340 nm (Habig et al., 1974, *J. Biol. Chem. 249*:7130-7139). GST fusion proteins produced from expression constructs derived from this expression vector can be purified by, *e.g.*, adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Another series of expression vectors of this type are the pBAD/His vectors (Guzman et al., *J. Bact. 177*:4121-4130, 1997; Invitrogen, Carlsbad, CA), which contains the following elements operably linked in a 5' to 3' orientation: the inducible, but tightly regulatable,

araBAD promoter; optimized *E. coli* translation initiation signals; an amino terminal polyhistidine(6xHis)-encoding sequence (also referred to as a "His-tag"); an XPRESS™ epitope-encoding sequence; an enterokinase cleavage site which can be used to remove the preceding N-terminal amino acids following protein purification, if so desired; a multiple cloning site; and an in-frame termination codon. Fusion proteins made from pBAD/His expression constructs can be purified using substrates or antibodies that specifically bind to the His-tag, and assayed by Western analysis using the Anti-Xpress™ antibody. Proteins made in such systems are designed to include heparin, thrombin, enterokinase, factor XA or other protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety by treatment with the appropriate protease.

Expression vectors derived from bacteriophage, including cosmids and phagemids, may also be used to express nucleic acids of interest in bacterial cells. Such vectors include, but are not limited to, Lambda FIX™, Lambda DASH™, Lambda ZAP™, Lambda EMBL3 and EMBL4 bacteriophage vectors, pBluescript™ phagemids, SuperCos and pWE15 vectors (all available from Stratagene) and the pSL1180 Superlinker Phagemid (Amersham Pharmacia Biotech).

In yeast such as *Saccharomyces cerevisiae* or *Pichia pastoris*, a number of vectors containing constitutive or inducible promoters such as those for mating factor alpha, *GAL1*, *TEF1*, *AOX1* or *GAP* may be used. Appropriate expression vectors include various pYES, pYD and pTEF derivatives (Invitrogen) (see, for example, Grant et al., *Methods in Enzymology 153*:516-544, 1987; Lundblad et al., Units 13.4 to 13.7 of Chapter 13 in: *Short Protocols in Molecular Biology*, 2nd Ed., Ausubel et al., eds., John Wiley & Sons, New York, New York, 1992, pages 13-19 to 13-33).

In cases where plant expression vectors are used, the expression of a nucleotide sequence of interest may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV (Brisson et al., *Nature 310*:511-514, 1984) may be used alone or in combination with the omega leader sequence from TMV (Takamatsu et al., *EMBO J. 6*:307-311, 1987). Alternatively, plant promoters such as the small subunit of RUBISCO (Coruzzi et al., *EMBO J.*

3:1671-1680, 1984; Broglie et al., *Science 224*:838-843, 1984); or heat shock promoters (Winter and Sinibaldi, *Results Probl. Cell. Differ. 17*:85-105, 1991) may be used. These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. For reviews of such techniques, see Gossen et al. (*Curr. Opin. Biotechnol. 5*:516-520, 1994), Porta and Lomonossoff (*Mol. Biotechnol. 3*:209-221, 1996) and Turner and Foster (*Mol. Biotechnol. 3*:225-36, 1995).

Another expression system which may be used to express a gene product of interest is an insect system. In one such system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. The nucleotide sequence of interest may be cloned into a nonessential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of the sequence of interest will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses are then used to infect *S. frugiperda* cells or *Trichoplusia* larvae in which the gene product of interest is expressed (see "Piwnica-Worms, Expression of Proteins in Insect Cells Using Baculovirus Vectors," Section II of Chapter 16 in: *Short Protocols in Molecular Biology*, 2nd Ed., Ausubel et al., eds., John Wiley & Sons, New York, New York, 1992, pages 16-32 to 16-48; López-Ferber et al., Chapter 2 in: *Baculovirus Expression Protocols*, Methods in Molecular Biology, Vol. 39, C.R. Richardson, Ed., Humana Press, Totawa, NJ, 1995, pages 25-63). *S. frugiperda* cells (Sf9, Sf21 or High Five™ cells) and appropriate baculovirus transfer vectors are commercially available from, *e.g.*, Invitrogen. Expression systems utilizing *Drosophila* S2 cells (also available from Invitrogen) may also be utilized.

Expression constructs for expressing nucleic acids of interest in mammalian cells are prepared in a stepwise process. First, expression cassettes that comprise a promoter (and associated regulatory sequences) operably linked to a nucleic acid of interest are constructed in bacterial plasmid-based systems; these expression cassette-comprising constructs are evaluated and optimized for their ability to produce the gene product of interest in mammalian cells that are transiently transfected therewith. Second, these expression cassettes are transferred to viral systems that

produce recombinant proteins during lytic growth of the virus (*e.g.*, SV40, BPV, EBV, adenovirus; see below) or from a virus that can stably integrate into and transduce a mammalian cellular genome (*e.g.*, a retroviral expression construct).

With regard to the first step, commercially available "shuttle" (*i.e.*, capable of replication in both *E. coli* and mammalian cells) vectors that comprise promoters that function in mammalian cells and can be operably linked to a nucleic acid of interest include, but are not limited to, SV40 late promoter expression vectors (*e.g.*, pSVL, Amersham Pharmacia Biotech), glucocorticoid-inducible promoter expression vectors (*e.g.*, pMSG, Amersham Pharmacia Biotech), Rous sarcoma enhancer-promoter expression vectors (*e.g.*, pRc/RSV, Invitrogen) and CMV early promoter expression vectors, including derivatives thereof having selectable markers to agents such as Neomycin, Hygromycin or ZEOCIN™ (*e.g.*, pRc/CMV2, pCDM8, pcDNA1.1, pcDNA1.1/Amp, pcDNA3.1, pcDNA3.1/Zeo and pcDNA3.1/Hygro, Invitrogen). In general, preferred shuttle vectors for nucleic acids of interest are those having selectable markers (for ease of isolation and maintenance of transformed cells) and inducible, and thus regulatable, promoters as overexpression of a gene product of interest may have toxic effects.

Methods for transfecting mammalian cells are known in the art (*see*, Kingston et al., "Transfection of DNA into Eukaryotic Cells," Section I of Chapter 9 in: *Short Protocols in Molecular Biology*, 2nd Ed., Ausubel et al., eds., John Wiley & Sons, New York, New York, 1992, pages 9-3 to 9-16). A control plasmid, such as pCH110 (Pharmacia), may be cotransfected with the expression construct being examined so that levels of the gene product of interest can be normalized to a gene product expressed from the control plasmid. Preferred expression cassettes, consisting essentially of a promoter and associated regulatory sequences operably linked to a nucleic acid of interest, are identified by the ability of cells transiently transformed with a vector comprising a given expression cassette to express high levels of the gene product of interest, or a fusion protein derived therefrom, when induced to do so. Expression may be monitored by Northern or Western analysis or, in the case of fusion

proteins, by a reporter moiety such as an enzyme or epitope. Effective expression cassettes are then incorporated into viral expression vectors.

Nucleic acids, preferably DNA, comprising preferred expression cassettes are isolated from the transient expression constructs in which they were prepared, characterized and optimized. A preferred method of isolating such expression cassettes is by amplification by PCR, although other methods (*e.g.*, digestion with appropriate restriction enzymes) can be used. Preferred expression cassettes are introduced into viral expression vectors, preferably retroviral expression vectors, in the following manner.

A DNA molecule comprising a preferred expression cassette is introduced into a retroviral transfer vector by ligation. Two types of retroviral transfer vectors are known in the art: replication-incompetent and replication-competent. Replication-incompetent vectors lack viral genes necessary to produce infectious particles but retain *cis*-acting viral sequences necessary for viral transmission. Such *cis*-acting sequences include the Ψ packaging sequence, signals for reverse transcription and integration, and viral promoter, enhancer, polyadenylation and other regulatory sequences. Replication-competent vectors retain all these elements as well as genes encoding virion structural proteins (typically, those encoded by genes designated *gag*, *pol* and *env*) and can thus form infectious particles in a variety of cell lines. In contrast, these functions are supplied in *trans* to replication-incompetent vectors in a packaging cell line, i.e, a cell line that produces mRNAs encoding *gag*, *pol* and *env* genes but lacking the Ψ packaging sequence. See, generally, Cepko, Unit 9.10 of Chapter 9 in: *Short Protocols in Molecular Biology*, 2nd Ed., Ausubel et al., eds., John Wiley & Sons, New York, New York, 1992, pages 9-30 to 9-35.

A retroviral construct comprising an expression cassette comprising a nucleic acid of interest produces RNA molecules comprising the cassette sequences and the Ψ packaging sequence. These RNA molecules correspond to viral genomes that are encapsidated by viral structural proteins in an appropriate cell line (by "appropriate" it is meant that, for example, a packaging cell line must be used for constructs based on replication-incompetent retroviral vectors). Infectious viral particles are then produced,

and released into the culture supernatant, by budding from the cellular membrane. The infectious particles, which comprise a viral RNA genome that includes the expression cassette for the gene product of interest, are prepared and concentrated according to known methods. It may be desirable to monitor undesirable helper virus, *i.e.*, viral particles which do not comprise the expression cassette for the gene product of interest. See, generally, Cepko, Units 9.11, 9.12 and 9.13 of Chapter 9 in: *Short Protocols in Molecular Biology*, 2nd Ed., Ausubel et al., eds., John Wiley & Sons, New York, New York, 1992, pages 9-36 to 9-45.

Viral particles comprising an expression cassette for the gene product of interest are used to infect *in vitro* (*e.g.*, cultured cells) or *in vivo* (*e.g.*, cells of a rodent, or of an avian species, which are part of a whole animal). Tissue explants or cultured embryos may also be infected according to methods known in the art. See, generally, Cepko, Unit 9.14 of Chapter 9 in: *Short Protocols in Molecular Biology*, 2nd Ed., Ausubel et al., eds., John Wiley & Sons, New York, New York, 1992, pages 9-45 to 9-48. Regardless of the type of cell used, production of the gene product of interest is directed by the recombinant viral genome.

In eukaryotic expression systems, host cells may be chosen for its ability to modulate the expression of the inserted sequences or, when the gene product of interest is a protein, to process the protein of interest in the desired fashion. Such modifications of proteins include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-translational processing which cleaves a "prepro" form of the protein of interest may also be important for its correct intracellular localization, folding and/or function. Different host cells such as CHO, HeLa, MDCK, HEK293, WI38, etc. have specific cellular machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of a protein of interest.

It may be desirable to use expression systems that can be tightly regulated, particularly in mammalian cells. By "tightly regulated" it is meant that the expression system is normally repressed (*i.e.*, kept from expressing the gene of interest) but can be induced to high levels of expression upon the addition of an inducing agent

to the cells harboring the expression construct. Such tightly regulated expression systems include, but are not limited to, ecdysone-inducible mammalian expression systems, tetracycline-regulated expression systems (such as the T-REx™ system, Invitrogen), and the GeneSwitch™ system (Invitrogen).

Expression systems of the invention also include the few systems in which a nucleic acid of interest is expressed from an organellar genome. Means for the genetic manipulation of the mitochondrial genome of *Saccharomyces cerevisiae* (Steele et al., *Proc. Natl. Acad. Sci. U.S.A. 93*:5253-5257, 1996) and systems for the genetic manipulation of plant chlorplasts (U.S. Patent No. 5,693,507; Daniell et al., *Nature Biotechnology 16*:345-348, 1998) have been described. Naturally, nucleic acids that encode polypeptide sequences have to be altered in organellar expression systems in order to reflect the differences in the genetic codes of organelles (see Table 1).

Genetic Modulation of Nucleic Acids and Gene Products

Various antisense-based methodologies may be used to modulate (reduce or eliminate) the expression of a nucleic acid of interest, and the corresponding gene product, in organelles, cells, tissues, organs and organisms. Such antisense modulation may be used to validate the role of a gene of interest in a disease or disorder or, when the causes or symptoms of a disease or disorder result from the over-expression of a nucleic acid of interest, as therapeutic agents.

The term "antisense" refers to nucleic acids that comprise one or more sequences that are the reverse complement of the "sense" strand of a gene, *i.e.*, the strand that is transcribed and, in the case of protein-encoding sequences, translated. Because antisense nucleic acids bind with high specificity to their targeted nucleic acids, selectivity is high and toxic side effects resulting from misdirection of the compounds can be minimal.

In general, antisense compositions are of two types: (i) synthetic antisense oligonucleotides, including enzymatic ones such as, *e.g.*, ribozymes; and (ii) antisense expression constructs. One skilled in the art will be able to utilize either modality as is appropriate to the given situation.

Synthetic antisense oligonucleotides are prepared from the reverse complement of a nucleic acid of interest. An antisense oligonucleotide consists of nucleic acid sequences corresponding to the reverse complement of a differentially expressed RNA. When introduced into cells expressing the RNA of interest, the antisense oligonucleotides specifically bind to the RNA molecules and interfere with their function by preventing secondary structures from forming or blocking the binding of regulatory or RNA-stabilizing factors. In addition, in the case of protein-encoding RNA species, oligonucleotides can inhibit RNA splicing, polyadenylation or protein translation, thus limiting or preventing the amount of protein made from such mRNAs. Additionally or alternatively, such oligoncuelotides can bind to double-stranded DNA molecules and form triplexes therewith, and thus interfere with the transcription of such sequences.

In instances where it is desired to target antisense oligonucleotides to RNAs produced from organellar genomes, peptide nucleic acids (PNAs) are preferred synthetic oligonucleotides. In PNAs, the sugar-phosphate backbone of biological nucleic acids has been replaced with a polypeptide-like chain. Targeting sequences that direct proteins to organelles can be conjugated to the backbone of antisense PNAs, with the result being that such conjugates are preferentially delivered to the targeted organelle (see, for example, published PCT applications WO 97/41150 and WO 99/05302, and Taylor et al., *Nature Genetics 15*:212-215, 1997).

Antisense oligonucleotides may be inherently enzymatic in nature, that is, capable of degrading the RNA molecule towards which they are targeted; such molecules are generally referred to as "ribozymes." A variety of increasingly short synthetic ribozyme frameworks that can be modified to comprise a nucleic acid sequence of interest have been described (Couture and Stinchcomb, *Trends Genet.* 12:510-515, 1996), including but not limited to hairpin ribozymes (Hampel, *Prog. Nucleic Acid Res. Mol. Biol. 58*:1-39, 1998), hammerhead ribozymes (Birikh et al., *Eur. J. Biochem. 245*:1-16, 1997) and minizymes (Kuwabara et al., *Nature Biotechnology 16*:961-965, 1998).

In the case of non-catalytic antisense nucleic acids in general, and ribozymes in particular, antisense modulation in a cell can also be achieved by expression constructs that direct the transcription of the reverse complement of a nucleotide sequence of interest *in vivo*. For example, in order to express non-catalytic antisense transcripts in mammalian or plant cells, all that may be required is the "flipping" (*i.e.*, reversing the orientation) of a nucleic acid of interest that has been cloned into a mammalian or plant expression vector, respectively. It is not necessary to maintain the proper relationship of elements such as translation signals and the like as the minimum requirement for an antisense expression construct of this type is a promoter operably linked to the reverse complement of a nucleic acid of interest. It is also possible to design expression constructs that express ribozymes in cells. Antisense and ribozyme expression constructs are also used to produce transgenic animals in which the level of expression of a gene of interest can be modulated in a temporal- or tissue-specific manner (see Sokol and Murray, *Transgenic Res.* 5:363-371, 1996, for a review).

Nucleic acid sequences derived according to the present invention may also be used to design "RNA decoys," *i.e.*, short RNA molecules corresponding to *cis*-acting regulatory sequences that bind *trans*-acting regulatory factors. When overexpressed in a cell or administered in excess thereto, such RNA decoys competitively inhibit the binding and thus action of the *trans*-acting regulatory factors, and thus limit or prevent the ability of such factors to carry out processes that stabilize (or destabilize) the RNA of interest, or enhance (or decrease) the polyadenylation, splicing nuclear transport, or translation of the RNA (Sullenger et al., *J. Virol.* 65:6811-6816, 1991). Expression of the RNA of interest may thus be either enhanced or decreased for therapeutic purposes.

Transgenic and Transmitochondrial Animals

Transgenic animals, modified with regard to a nucleic acid of interest, may be prepared. Such animals are useful for developing animal models of human disease and for evaluating the safety and effectiveness of therapeutic agents of the

invention. In general, such transgenic animals are of three types: (i) "transgenic knock-outs," in which the animal's homolog of a gene of interest is disrupted or removed, with a resulting more-or-less total loss of function of the corresponding gene product; (ii) "regulatable transgenics," in which the gene of interest is operably linked to an inducible promoter; and (iii) "replacement transgenics," in which the animal's homolog of the gene of interest has been replaced with the human gene of interest, which may be expressed from an endogenous or inducible promoter.

The non-human transgenic animals of the invention comprise any animal that can be genetically manipulated to produce one or more of the above-described classes of transgenic animals. Such non-human animals include vertebrates such as rodents, non-human primates, sheep, dog, cow, amphibians, reptiles, etc. Preferred non-human animals are selected from non-human mammalian species of animals, including without limitation animals from the rodent family including but not limited to rats and mice, most preferably mice (see, *e.g.*, U.S. Patents 5,675,060 and 5,850,001). Other non-human transgenic animals that may be prepared include without limitation rabbits (U.S. Patent No. 5,792,902), pigs (U.S. Patent No. 5,573,933), bovine species (U.S. Patents 5,633,076 and 5,741,957) and ovine species such as goats and sheep (U.S. Patents 5,827690; 5,831,141; and 5,849,992).

The transgenic animals of the invention are animals into which has been introduced by non-natural means (*i.e.*, by human manipulation), one or more genes that do not occur naturally in the animal, *e.g.*, foreign genes, genetically engineered endogenous genes, etc. The non-naturally introduced genes, known as transgenes, may be from the same or a different species as the animal but not naturally found in the animal in the configuration and/or at the chromosomal locus conferred by the transgene. Transgenes may comprise foreign DNA sequences, *i.e.*, sequences not normally found in the genome of the host animal. Alternatively or additionally, transgenes may comprise endogenous DNA sequences that are abnormal in that they have been rearranged or mutated *in vitro* in order to alter the normal *in vivo* pattern of expression of the gene, or to alter or eliminate the biological activity of an endogenous gene product encoded by the gene. (Watson et al., in *Recombinant DNA*, 2d Ed., W.H.

Freeman & Co., New York, 1992), pages 255-272; Gordon, *Intl. Rev. Cytol. 115*:171-229, 1989; Jaenisch, *Science 240*:1468-1474, 1989; Rossant, *Neuron 2*:323-334, 1990).

The transgenic non-human animals of the invention are produced by introducing transgenic constructs comprising sequences of interest, or the host animal's homologs thereof, into the germline of the non-human animal. Embryonic target cells at various developmental stages are used to introduce the transgenes of the invention. Different methods are used depending on the stage of development of the embryonic target cell(s).

Microinjection of zygotes is the preferred method for incorporating transgenes into animal genomes in the course of practicing the invention. A zygote, a fertilized ovum that has not undergone pronuclei fusion or subsequent cell division, is the preferred target cell for microinjection of transgenic DNA sequences. The murine male pronucleus reaches a size of approximately 20 micrometers in diameter, a feature which allows for the reproducible injection of 1-2 picoliters of a solution containing transgenic DNA sequences. The use of a zygote for introduction of transgenes has the advantage that, in most cases, the injected transgenic DNA sequences will be incorporated into the host animal's genome before the first cell division (Brinster et al., *Proc. Natl. Acad. Sci. U.S.A. 82*:4438-4442, 1985). As a consequence, all cells of the resultant transgenic animals (founder animals) stably carry an incorporated transgene at a particular genetic locus, referred to as a transgenic allele. The transgenic allele demonstrates Mendelian inheritance: half of the offspring resulting from the cross of a transgenic animal with a non-transgenic animal will inherit the transgenic allele, in accordance with Mendel's rules of random assortment.

Viral integration can also be used to introduce the transgenes of the invention into an animal. The developing embryos are cultured *in vitro* to the developmental stage known as a blastocyte. At this time, the blastomeres may be infected with appropriate retroviruses (Jaenisch, *Proc. Natl. Sci. U.S.A. 73*:1260-1264, 1976; Soriano and Jaenisch, *Cell 46*:19-29, 1986). Infection of the blastomeres is enhanced by enzymatic removal of the zona pellucida (Hogan, et al., in *Manipulating the Mouse Embryo*, Cold Spring Harbor Press, Cold Spring Harbor, N.Y., 1986).

Transgenes are introduced via viral vectors which are typically replication-defective but which remain competent for integration of viral-associated DNA sequences, including transgenic DNA sequences linked to such viral sequences, into the host animal's genome (Jahneret al., *Proc. Natl. Acad. Sci. U.S.A. 82*:6927-6931, 1985; Van der Putten et al., *Proc. Natl. Acad. Sci. U.S.A. 82*:6148-6152, 1985). Transfection is easily and efficiently obtained by culture of blastomeres on a mono-layer of cells producing the transgene-containing viral vector (Van der Putten et al., *Proc. Natl. Acad. Sci. U.S.A. 82*:6148-6152, 1985; Stewart, et al., *EMBO J. 6*:383-388, 1987). Alternatively, infection may be performed at a later stage, such as a blastocoele (Jahneret al., *Nature 298*:623-628, 1982). In any event, most transgenic founder animals produced by viral integration will be mosaics for the transgenic allele; that is, the transgene is incorporated into only a subset of all the cells that form the transgenic founder animal. Moreover, multiple viral integration events may occur in a single founder animal, generating multiple transgenic alleles which will segregate in future generations of offspring. Introduction of transgenes into germline cells by this method is possible but probably occurs at a low frequency (Jahner et al., *Nature 298*:623-628, 1982). However, once a transgene has been introduced into germline cells by this method, offspring may be produced in which the transgenic allele is present in all of the animal's cells, *i.e.*, in both somatic and germline cells.

Embryonic stem (ES) cells can also serve as target cells for introduction of the transgenes of the invention into animals. ES cells are obtained from pre-implantation embryos that are cultured *in vitro* (Evans et al., *Nature 292*:154-156, 1981; Bradley et al., *Nature 309*:255-258, 1984; Gossler et al., *Proc. Natl. Acad. Sci. U.S.A. 83*:9065-9069, 1986; Robertson et al., *Nature 322*:445-448, 1986; Robertson, E.J., in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, Robertson, E.J., ed., IRL Press, Oxford, 1987, pp. 71-112). ES cells, which are commercially available (from, *e.g.*, Genome Systems, Inc., St. Louis, MO), can be transformed with one or more transgenes by established methods (Lovell-Badge, R.H., in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, Robertson, E.J., ed., IRL Press, Oxford, 1987, pp. 153-182). Transformed ES cells can be

combined with an animal blastocyst, whereafter the ES cells colonize the embryo and contribute to the germline of the resulting animal, which is a chimera (composed of cells derived from two or more animals) (Jaenisch, *Science 240*:1468-1474, 1988; Bradley in: *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, Robertson, E.J., ed., IRL Press, Oxford 1987, pp. 113-151). Again, once a transgene has been introduced into germline cells by this method, offspring may be produced in which the transgenic allele is present in all of the animal's cells, *i.e.*, in both somatic and germline cells.

However it occurs, the initial introduction of a transgene is a Lamarckian (non-Mendelian) event. However, the transgenes of the invention may be stably integrated into germ line cells and transmitted to offspring of the transgenic animal as Mendelian loci. Other transgenic techniques result in mosaic transgenic animals, in which some cells carry the transgenes and other cells do not. In mosaic transgenic animals in which germ line cells do not carry the transgenes, transmission of the transgenes to offspring does not occur. Nevertheless, mosaic transgenic animals are capable of demonstrating phenotypes associated with the transgenes.

Offspring that have inherited the transgenes of the invention are distinguished from littermates that have not inherited transgenes by analysis of genetic material from the offspring for the presence of biomolecules that comprise unique sequences corresponding to sequences of, or encoded by, the transgenes of the invention. For example, biological fluids that contain polypeptides uniquely encoded by the transgenes of the invention may be immunoassayed for the presence of the polypeptides. A more simple and reliable means of identifying transgenic offspring comprises obtaining a tissue sample from an extremity of an animal, *e.g.*, a tail, and analyzing the sample for the presence of nucleic acid sequences corresponding to the DNA sequence of a unique portion or portions of the transgenes of the invention. The presence of such nucleic acid sequences may be determined by, *e.g.*, hybridization ("Southern") analysis with DNA sequences corresponding to unique portions of the transgene, analysis of the products of PCR reactions using DNA sequences in a sample as substrates and oligonucleotides derived from the transgene's DNA sequence, *etc.*

Cloned animals, transgenic and otherwise, of the invention may also be prepared (for a review of mammalian cloning techniques, see Wolf et al., *J. Assist. Reprod. Genet. 15*:235-239, 1998). Such cloned animals include, without limitation, ovine species such as sheep (Campbell et al., *Nature 380*:64-66, 1996; Wells et al., *Biol. Reprod. 57*:385-393, 1997) rodents such as mice (Wakayama et al., *Nature 394*:369-374, 1998) and non-human primates such as rhesus monkeys (Meng et al., *Biol. Reprod. 57*:454-459, 1997).

The transgenic and cloned animals of the invention may be used as animal models of human disease states and to evaluate potential therapies for such disease states. For example, in such methods, a first transgenic animal having a disease state (or one or more symptomatic components thereof) is given a known dose of a candidate therapeutic composition or exposed to a candidate therapeutic treatment, and a second (control) transgenic animal is given a placebo or not exposed to the candidate therapeutic treatment. Symptoms and/or clinical end-points relevant to the disease state are measured in both animals over appropriate intervals of time, and the results are compared. Therapeutic (desirable) compositions and treatments are identified as those which ameriolate, delay the onset of or eliminate such symptoms and end-points in the treated animal relative to the control animal. In like fashion, undesirable compositions and treatments that aggravate or accelerate the disease state are identified as those which enhance the degree of such symptoms and end-points and/or hasten their onset. Because of their high degree of genetic identity, cloned transgenic animals are preferred in such methods.

With regard to transmitochondrial animals, two types of such animals presently exist. First, because of the way they are generated ("nuclear transfer"), "Dolly-like" cloned animals are cybrid-like transmitochondrial animals. In nuclear transfer, a donor somatic cell is electrofused with a recipient enucleated oocyte; this method was used to produce Dolly, the first mammal reported to have been cloned (Wilmut et al., *Nature 385*:810-813, 1997). When the mitochondrial DNA (mtDNA) in Dolly and in nine other nuclear transfer-derived sheep generated from fetal cells was examined, it was found that the mtDNA of each of the ten nuclear-transfer sheep was

derived exclusively from recipient enucleated oocytes. There was no detectable contribution of mtDNA from the respective somatic donor cells. Thus, although these ten sheep are authentic nuclear clones, they are in fact "cybrid animals", containing mtDNA that is (apparently) derived from the oocyte, and nuclear DNA derived from the somatic cells used in the cloning process (Evans et al., *Nature Genetics 23*:90-93, 1999).

A second type of transmitochondrial animal is a heteroplasmic animal, *i.e.*, one that has been manipulated so that the animal contains mitochondrial genomes from two or more animals. Such animals may (or may not) contain heteroplasmic cells in which two different mitochondrial genomes are contained, and/or may be chimeric with regard to their heteroplasmy (*i.e.*, some cells contain only a first mitochondrial genome, whereas other cells only contain a second mitochondrial genome.

In any event, heteroplasmic transmitochondrial animals can be generated in at least two ways. In one method of generating heteroplasmic transmitochondrial animals, purified mitochondria from a first animal having one mitchondrial genome are micro-injected into ova derived from a second animal having a different mitochondrial genome, and the manipulated ova are then implanted into pseudopregnant mice (see Pinkert et al., *Transgenic Research 6*:379-383, 1997; Irwin et al., *Transgenic Research 8*:119-123, 1999; and WO 99/05259). In a second method of generating heteroplasmic transmitochondrial animals, one-cell embryos of one strain of animal are electrofused to cytoplasts recovered from zygotes of another strain of animal (Jenuth et al., *Nature Genetics 14*:146-151, 1996).

Polypeptides and Proteins

The nucleic acids of interest identified according to the methods of the invention may encode amino acid sequences. Such amino acid sequences may correspond to a full-length protein or to a polypeptide portion thereof.

In instances wherein a full-length protein is encoded by a nucleic acid of interest, the protein may be a known protein that is commercially available or one to which antibodies are known and can be used to isolate the protein from appropriate

biological samples. If a full-length protein of the invention has not previously been described, it may be produced via recombinant DNA methodologies or prepared from biological samples using known biochemical techniques. Short (*i.e.*, having less than about 30 amino acids) polypeptides that are encoded by short (*i.e.*, having less than about 100 nucleotides) nucleic acids of the invention or derived from the amino acid sequences encoded by longer nucleic acids or from full-length proteins can be synthesized *in vitro* by methods known in the art. Fusion proteins comprising amino acid sequences of interest may also be prepared and are included within the scope of the polypeptides and proteins of the invention.

Regardless of the means by which they are prepared, the polypeptides and proteins of the invention have a variety of applications. They may be used to generate antibodies or to screen for ligands that may serve as therapeutic agents, or may themselves be used as therapeutic agents. Full-length proteins of the invention may have the activity of the wildtype protein and may thus be used to treat conditions resulting from a loss of such activity. Polypeptides of the invention may also have such activities, or may competitively inhibit a protein of interest *in vivo* by binding a ligand of the protein. If the ligand is an activator of the protein, such polypeptides may be used to treat conditions resulting from the over-expression or over-activation of the protein *in vivo*. If the ligand is a toxin or activator of cell death (apoptosis or necrosis), administration of a protein or polypeptide that binds such a ligand to a patient in need thereof will have the beneficial effect of competitively inhibiting the action of the toxin or cell death activator.

Antibodies

Antibodies to a protein or polypeptide of interest are prepared according to a variety of methods known in the art. In general, such antibodies may be polyclonal, monoclonal or monospecific antibodies. Primary antibodies of the invention bind specifically to a particular protein or polypeptide of interest and are thus used in assays to detect and quantitate such proteins and polypeptides. In such assays, generally referred to in the art as immunoassays, a primary antibody of the invention is detectably

labeled or is specifically recognized and monitored by a detectably labeled secondary antibody or a combination of a secondary antibody and a tertiary molecule (which may also be an antibody) that is detectably labeled. Regardless of the specific format, the primary antibody of the invention provides a means by which a protein or polypeptide of interest is specifically bound and subsequently detected. One preferred assay format is the Enzyme-Linked Immunosorbent Assay (ELISA) format.

A nucleic acid of interest may encode a known protein or a portion thereof, or a polypeptide sequence that is homologous to a known protein. In such instances, antisera to the known protein, or the known protein itself, may be commercially available. In the latter instance, or when the nucleic acid of interest can be used to produce a protein of interest (or a polypeptide portion thereof greater than about 30 amino acids in length) via recombinant DNA expression techniques, the known or recombinantly-produced protein can be used to immunize a mammal of choice (*e.g.*, a rabbit, mouse or rat) in order to produce antisera from which polyclonal antibodies can be prepared (see, *e.g.*, Cooper and Paterson, Units 11.12 and 11.13 in Chapter 11 in: *Short Protocols in Molecular Biology*, 2nd Ed., Ausubel et al., eds., John Wiley & Sons, New York, New York, 1992, pages 11-37 to 11-41).

In the event that a nucleic acid sequence of interest encodes a polypeptide sequence for which no complete protein (or homolog thereof) is known, is too short to encode more than about 30 amino acids (*i.e.*, the nucleic acid of interest is less than about 100 nucleotides in length), or encodes more than one polypeptide sequence of potential interest, such candidate amino acid sequences can be used to synthesize one or more polypeptide molecules, each of which has a defined amino acid sequence. Such synthetic polypeptides can then be used to immunize animals (*e.g.*, rabbits) according to methods known in the art (Collawn and Paterson, Units 11.14 and 11.15 in Chapter 11 in: *Short Protocols in Molecular Biology*, 2nd Ed., Ausubel et al., eds., John Wiley & Sons, New York, New York, 1992, pages 11-42 to 11-46; Cooper and Paterson, Units 11.12 and 11.13 in Chapter 11 in: *Short Protocols in Molecular Biology*, 2nd Ed., Ausubel et al., eds., John Wiley & Sons, New York, New York, 1992, pages 11-37 to 11-41). The resulting antisera, which is specific for a particular peptide

and is sometimes referred to as "monospecific," may then be used to probe cells from which the nucleic acid of interest was isolated. A positive response to a given antiserum indicates that the candidate reading frame from which the synthetic polypeptide used to raise the antiserum was derived is a reading frame used to encode at least one protein in the cell(s) so examined. Moreover, such an antiserum can be used to identify proteins of interest in the cells from which the nucleic acid of interest was isolated.

Because of their high degree of specificity and homogeneity, monoclonal antibodies are often the preferred type of antibody for a variety of applications. Methods for producing and preparing monoclonal antibodies are known in the art (see, *e.g.*, Fuller et al., Units 11.4 to 11.11 in Chapter 11 in: *Short Protocols in Molecular Biology*, 2nd Ed., Ausubel et al., eds., John Wiley & Sons, New York, New York, 1992, pages 11-22 to 11-36). Murine monoclonal antibodies may be "humanized" and used as therapeutic agents (see, *e.g.*, Güssow and Seemann, *Methods in Enzymology 203*:99-121, 1991; Vaughan et al., *Nature Biotechnology 16*:535-539, 1998).

Antibodies to proteins and polypeptides of interest are used to detect such proteins and polypeptides in a variety of assay formats. Such immunoassays may useful in diagnostic, prognostic or pharmacogenomic methods of the invention, or in methods in which various cell types, tissues or organs are probed for the presence of a protein of interest. Monoclonal antibodies are generally preferred for such methods due to their high degree of specificity and homogeneity.

Diagnostic Prognostic and Pharmacogenomic Methods

Assays for or utilizing one or more of the antibodies, polypeptides and proteins, ligands therefor and nucleic acid probes and primers of the invention are used in diagnostic, prognostic and pharmacogenomic methods of the invention. The term "diagnostic" refers to assays that provide results which can be used by one skilled in the art, typically in combination with results from other assays, to determine if an individual is suffering from a disease or disorder of interest, whereas the term "prognostic" refers to the use of such assays to evaluate the response of an individual

having such a disease or disorder to therapeutic or prophylactic treatment. The term "pharmacogenomic" refers to the use of assays to predict which individual patients in a group will best respond to a particular therapeutic or prophylactic composition or treatment.

The terms "disease" and "disorder" refer without limitation to illnesses and abnormal conditions resulting from infection by one or more pathogens or parasites, exposure to toxic compounds or harmful physical conditions, genetic deficiencies such as inborn errors of metabolism, hyperproliferative diseases such as tumors and cancers, auto-immune disorders, psychological and metal disorders, undesirable results of the aging process, inabilities to perform sexual activities, damage resulting from physical trauma or environmental conditions and the like. Neither disease nor disorder encompasses pregnancy *per se* but certain diseases and disorders may particularly impact pregnant individuals or fetuses and embryos.

In diagnostic applications of the invention, samples from individuals are assayed with regard to the relative or absolute amounts of a "marker," *i.e.*, a nucleic acid or protein of interest, or an endogenous ligand of or antibody to a nucleic acid or protein of interest. An increased or decreased level of a marker relative to control levels indicates that the individual from which the sample was taken has, has had, or is likely to develop the disease or disorder of interest. The term "control level" refers to the level of marker present in samples taken from one or more individuals known to not have the disease or disorder of interest, or to the level of marker present in a sample taken from the individual in question before of after the diagnostic sample. Additionally or alternatively, a number of individuals known to not have the disease or disorder of interest are tested for levels of the marker, and an absolute amount or concentration corresponding to a normal level of the marker is established; in this embodiment, effected individuals are identified as those having a level of marker that is significantly lower or higher than the normal value.

In prognostic applications of the invention, samples from individuals are assayed as in the preceding paragraph, but (i) the individuals in question are known to be suffering from the disease or disorder of interest and (ii) the results of the assays are

put to a related but different use. Specifically, such assays are used to evaluate the response of an individual having a disease or disorder to therapeutic or prophylactic treatment, and to predict the course of recovery therefrom or to determine the need for additional or alternative treatments.

In pharmacogenomic applications of the invention, patients suffering from a disease or disorder of interest are stratified with regard to desirable or undesirable responses using one or more assays of the invention. A therapeutic composition and/or treatment known to be more effective, or which produces more side-effects, in some patients as compared to others is administered a group of patients suffering from a disease or disorder of interest. A method of identifying which patients having the disease are more likely to respond to a therapeutic composition and/or treatment comprises providing samples from a group of patients having the disease; measuring the amount of a protein or polypeptide of interest, or of a nucleic acid of interest, or a ligand therefor or antibody thereto, present in the samples; providing the therapeutic composition and/or treatment to the patients; measuring the degree, frequency, rate or extent of responses of the patients to the therapeutic composition and/or treatment; and determining if a correlation exists between the amount of amount of the protein or polypeptide of interest, or of a nucleic acid of interest, or a ligand therefor or antibody thereto present in the samples and the degree, frequency, rate or extent of such responses.

The resulting correlations are used to stratify patients in the following manner. If such a correlation is a positive correlation, the presence of such correlation indicates that patients yielding samples having an increased amount of the protein or polypeptide of interest, or the ligand therefor, or of the nucleic acid of interest are more likely to respond to the treatment. In contrast, if the correlation is a negative correlation, the presence of the correlation indicates that patients yielding samples having an increased amount of the protein or polypeptide of interest, or the ligand therefor, or of the nucleic acid of interest are less likely to respond to the treatment.

The response(s) that are measured in these methods can be desirable response(s), in which case it is preferred to provide the therapeutic composition and/or

treatment to patients having a relatively high level of the protein or polypeptide of interest, or the ligand therefor, or of the nucleic acid of interest present. Alternatively, the response(s) that are measured in these methods can be undesirable response(s), in which case it is preferred to avoid providing the therapeutic composition and/or treatment to patients having a relatively high level of the protein or polypeptide of interest, or the ligand therefor, or of the nucleic acid of interest.

The assays for the preceding methods may be performed at a laboratory to which patient-derived samples or delivered, or at the site of patient treatment. In the latter instance, kits for performing one or more assays of the invention are preferred. Antibodies, polypeptides and proteins, ligands therefor and nucleic acid probes and primers of the invention can be provided in kit form, *e.g.*, in a single or separate container, along with other reagents, buffers, enzymes or materials to be used in practicing at least one method of the invention. Such kits can be provided in a container that can optionally include instructions or software for performing a method of the invention. Such instructions or software can be provided in any language or human- or machine-readable format.

Compound Screening, including High-Throughput Assays

The nucleic acids, proteins, polypeptides, antibodies and transgenic animals of the invention may be used to validate the role of a gene product of interest in a particular disease, disorder or undesirable response, and to screen for conditions or compounds that can be used to treat such diseases, disorders and undesirable responses, preferably using high-throughput screening methods such as they are known in the art or later developed. Such treatment can be remedial, therapeutic, palliative, rehabilitative, preventative, impeditive or prophylactic in nature. Diseases and disorders to which the invention may be applied, including organellar associated diseases as provided herein, include without limitation, mitochondria associated diseases, including but not limited to neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD); auto-immune diseases; diabetes mellitus, including Type I and Type II; MELAS, MERFF, arthritis, NARP (Neuropathy; Ataxia;

Retinitis Pigmentosa); MNGIE (Myopathy and external ophthalmoplegia; Neuropathy; Gastro-Intestinal; Encephalopathy), LHON (Leber's; Hereditary; Optic; Neuropathy), Kearns-Sayre disease; Pearson's Syndrome; PEO (Progressive External Ophthalmoplegia); congenital muscular dystrophy with mitochondrial structural abnormalities; Wolfram syndrome (DIDMOAD; Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy, Deafness), Leigh's Syndrome, fatal infantile myopathy with severe mtDNA depletion, benign "later-onset" myopathy with moderate reduction in mtDNA; dystonia; schizophrenia; mitochondrial encephalopathy, lactic acidosis, and stroke (MELAS); mitochondrial diabetes and deafness (MIDD); myoclonic epilepsy ragged red fiber syndrome (MERFF); and hyperproliferative disorders, such as cancer, tumors and psoriasis.

The term "undesirable response" refers to a biological or biochemical response by one or more cells of an organism to one or more physical conditions, chemical agents, or combinations thereof that leads to an undesirable consequence. An undesirable response can occur at the organellar level (*e.g.*, loss of $\Delta\psi$ in mitochondria, inhibition of photosynthesis in chloroplasts), the cellular level (*e.g.*, cell death such as apoptosis or necrosis), in tissues (*e.g.*, ischemia), in organs (*e.g.*, ischemic heart disease) or to the organism as a whole (*e.g.*, death; loss of reproductive capacity or cognitive processes).

Physical conditions that may produce an undesirable response include, without limitation, hypothermia, hyperthermia, dehydration, exposure to ultraviolet and other types of radiation, micro-gravity, physical trauma, tensile stress, and exposure to electrical or magnetic fields. Chemical agents that may produce an undesirable response include without limitation reactive oxygen species (ROS), apoptogens, and the like.

Nucleic acids of the invention are used to screen for conditions or compounds that can be used to treat disease states and undesirable responses in the following manner. Treatment of cells with antisense molecules, including ribozymes, or introduction therein of antisense constructs, specific for a given gene product of interest should result in such cells demonstrating at least one of the biochemical or

biological defects associated with the disease or disorder for which the gene product is being validated. In like fashion, transgenic animals comprising constructs directing the over-expression of a gene of interest, or an antisense or ribozyme expression construct, or animals to which antisense, ribozyme or molecular decoy oligonucleotides are administered, will demonstrate at least one of the biochemical or biological defects associated with the disease or disorder of interest if the nucleic acid encodes a gene product that is a valid target for the disease or disorder.

Similarly, for proteins of interest that may be targets for therapeutic intervention, cells may be contacted with one or more antibodies specific for the protein, and the presentation of responses associated with the disease or disorder will be seen with valid targets. Polypeptides and proteins of the invention are also used to screen for conditions or compounds that can be used to treat disease states and undesirable responses in the following manner. The protein of interest, or a polypeptide derived therefrom having at least one activity of the protein of interest, is produced by recombinant DNA methods or *in vitro* synthetic techniques. The protein or polypepeptide, which may be attached to a solid support, is contacted with a detectably labeled ligand (including, for example, an antibody). A compound is then introduced to the reaction vessel, and active compounds are identified as those that cause the release of the detectably labeled ligand.

Therapeutic Applications

Therapeutic agents derived therefrom according to the above embodiments can be employed in combination with conventional excipients, *i.e.*, pharmaceutically acceptable organic or inorganic carrier substances suitable for parenteral application which do not deleteriously react with the active compound. Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions, alcohol, vegetable oils, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid monoglycerides and diglycerides, petroethral fatty acid esters, hydroxymethylcellulose, polyvinylpyrrolidone, etc. The pharmaceutical preparations can be sterilized and if

desired, mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavoring and/or aromatic substances and the like which do not deleteriously react with the active compounds. For parenteral application, particularly suitable vehicles consist of solutions preferably oily or aqueous solutions, as well as suspensions, emulsions, or implants. Aqueous suspensions may contain substances which increase the viscosity of the suspension and include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers (see generally WO 98/13353 to Whitney, published April 2, 1998).

The term "therapeutically effective amount," for the purposes of the invention, refers to the amount of a therapeutic agent which is effective to achieve its intended purpose. While individual needs vary, determination of optimal ranges for effective amounts of a therapeutic agent is within the skill of the art. Human doses can be extrapolated from animal studies (Fingle and Woodbury, Chapter 1 in *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 5th Ed., MacMillan Publishing Co., New York (1975), pages 1-46). Generally, the dosage required to provide an effective amount of the composition, and which can be adjusted by one of ordinary skill in the art will vary, depending on the age, health physical condition, weight, extent of disease of the recipient, frequency of treatment and the nature and scope of the desired effect.

Therapeutic agents of the invention can be delivered to mammals via intermittent or continuous intravenous injection of one or more these compositions or of a liposome (Rahman and Schein, in *Liposomes as Drug Carriers*, Gregoriadis, ed., John Wiley, New York (1988), pages 381-400; Gabizon, A., in *Drug Carrier Systems*, Vol. 9, Roerdink et al., eds., John Wiley, New York, 1989, pp. 185-212) or microparticle (Tice et al., U.S. Patent 4,542,025) formulation comprising one or more of these compositions; via subdermal implantation of drug-polymer conjugates (Duncan, *Anti-Cancer Drugs 3*:175-210, 1992; via microparticle bombardment (Sanford et al., U.S. Patent 4,945,050); via infusion pumps (Blackshear and Rohde, in: *Drug Carrier Systems*, Vol. 9, Roerdink et al., eds., John Wiley, New York, 1989, pp. 293-310) or by

other appropriate methods known in the art (see, generally, *Remington's Pharmaceutical Sciences*, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, PA, 1990). Anti-cancer therapeutic compositions of the invention may be used in combination with other anti-cancer compositions known in the art.

ASPECTS OF THE INVENTION

I. Identification of Differentially Expressed Organellar Factors

It is an object of the invention to identify organellar factors encoded by genes that are differentially expressed in particular disease states, apoptosis, in response to various stressors or in a species-specific fashion. By "differentially expressed," it is meant that the gene product is present in greater amounts in one cell type, or under one set of conditions, than in another.

Organellar factors may be macromolecules found within or associated with organelles, or cellular factors that negatively or positively influence, either directly or indirectly, the amount and/or activity of such macromolecules. Such factors include gene products that are expressed from genes that are derived from a cell's or organism's nuclear genome, as well as those expressed from the genomes of organelles such as mitochondria or chloroplasts. Nuclear genomes and genes may include organellar "pseudogene" sequences, *i.e.*, sequences originally present in organellar genomes that have been translocated from the organellar genome to the nuclear genome. Pseudogene sequences are generally not normally expressed but may become active in certain disease states or in response to certain conditions such as, *e.g.*, cellular stress.

A gene product may be a RNA molecule or a protein. Of particular interest are those genes and gene products that are differentially expressed in a disease state (*i.e.*, differentially expressed in cells from a diseased organism relative to cells from an undiseased, control organism of the same species), in manipulated cells versus wildtype cells, or in a species-specific manner (*i.e.*, differentially expressed in cells from one species relative to cells from a second species). Thus, for example, an "RNA of interest," a "gene of interest" and a "protein of interest" refer to, respectively, a RNA, gene and protein that are differentially expressed with regard to a disease state, in

manipulated cells or in a species-specific manner. As one example of a gene of interest that does not directly encode a mitochondrial gene product, a nucleic acid of interest may be an antisense regulator of a mitochondrial gene product (Shayiq, J. Biol. Chem. 272:4050-4057 (1997)). "RNAs of interest" include RNA molecules that are not mRNA molecules but are themselves gene products such as, for example, ribosomal RNA (rRNA) molecules, transfer RNA (tRNA) molecules, ribozymes, RNA molecules that form part of a nucleoprotein complex, and antisense transcripts.

As regards genes and gene products that are differentially expressed in a disease or disorder, "mitochondria associated disorders," *i.e.*, diseases associated or thought to be associated with altered mitochondrial function and/or mitochondrial mutations, are of particular interest. Mitochondria associated disorders may include without limitation AD, PD, auto-immune diseases, diabetes mellitus, MELAS, MERFF, arthritis, NARP (Neuropathy; Ataxia; Retinitis Pigmentosa); MNGIE (Myopathy and external ophthalmoplegia; Neuropathy; Gastro-Intestinal; Encephalopathy), LHON (Leber's; Hereditary; Optic; Neuropathy), Kearns-Sayre disease; Pearson's Syndrome; PEO (Progressive External Ophthalmoplegia); congenital muscular dystrophy with mitochondrial structural abnormalities; Wolfram syndrome (DIDMOAD; Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy, Deafness), Leigh's Syndrome, fatal infantile myopathy with severe mtDNA depletion, benign "later-onset" myopathy with moderate reduction in mtDNA; dystonia; schizophrenia; mitochondrial encephalopathy, lactic acidosis, and stroke (MELAS); mitochondrial diabetes and deafness (MIDD); myoclonic epilepsy ragged red fiber syndrome (MERFF); and hyperproliferative disorders, such as cancer, tumors and psoriasis.

One aspect of the present invention is a method for identifying organellar factors encoded by genes that are differentially expressed, comprising: providing one or more cells in a first state, providing one or more cells in a second state, determining the expression of genes in the first state and the second state, and identifying genes or proteins that are differentially expressed in the first state and the second state.

The cell(s) in the first state and the cell(s) in the second state can be the same or different and can be any cell or population of cells, such as a primary cell line,

a continuous cell line, a population of clones, a population of cells, a manipulated cell line, a population of manipulated cells, or a cell or population of cells derived from the same or different organism or species of organism, such as a sample, fluid, tissue or organ, or any combination of the foregoing. "Derived from," as used in this context, refers to cells whose lineage can be traced to a taxonomical kingdom, phylum, class or order; preferably a family of genus; and more preferably a species, and most preferably an identified organism. An organism can be a transmitochondrial organism, a transgenic organism or a non-transgenic organism. Reference to an organism refers to a particular organism or a group of organisms. When a group of organisms is used in a method of the present invention, the organisms can be from the same species, but that need not be the case.

The first state and the second state can be different regarding a particular disease state. For example, the cell(s) in the first state can be derived from a first organism having a diseased state and the cell(s) in the second state can be derived from a second organism not having the diseased state or from a normal organism. For example, the cell(s) in the first state can be from a patient diagnosed as having Alzheimer's disease and the cell(s) in the second state can be from a patient not being diagnosed as having Alzheimer's disease.

In addition, the first and second states can be different based on the different source of the sample, fluid, tissue or organ. In this aspect of the invention, the cell(s) in the first state can be derived from a different sample, fluid, tissue or organ as the cell(s) in the second state. For example, the cell(s) in the first state can be one or more muscle cells and the cell(s) in the second state can be one or more central nervous system cells.

Furthermore, the first state and the second state can be different based on the different treatments or the course of treatments of at least one organism. In this aspect of the present invention, the cell(s) in the first state can be derived from the same or different organism provided a treatment of a course of treatment, such as environment, diet, or administration of compounds, such as proteins, peptides, nucleic acids (such as in a vector, such as a viral vector), drugs, chemicals or toxins, as the

cell(s) in the second state is (are) derived from. A sample, fluid, tissue or organ can be taken at different times over the course of such treatment from one or more organisms that receive a treatment, do not receive a treatment or receive a different treatment. These samples, fluids, tissues or organs can be the source of the cell(s) in the first state or the cell(s) in the second state. For example, the cell(s) in the first state can be derived from an organism before being provided a treatment and the cell(s) in the second state can be derived from the same or different organism at different times during such treatment. By way of further example, the cell(s) in the first state can be derived from an organism receiving a first treatment and the cell(s) in the second state can be derived from a different organism receiving a second treatment.

In addition, the first state and the second state can be different based on treatment of at least one of the cell(s) in the first state or the cell(s) in the second state with at least one compound. For example, the cell(s) in the first state can be treated with a compound, such as a protein, peptide, nucleic acid (such as in a vector, such as a viral vector), drug, chemical or toxin and the cell(s) in the second state not be treated with the compound used to treat the at least one first cell, be treated with a compound different from the compound used to treat the cell(s) in the first state, or be treated with the compound used to treat the cell(s) in the first state but at a different concentration.

Furthermore, the first state and the second state can be different based on the presence of one or more cellular stressors. The cellular stressor(s) can be any cellular stressor, but is preferably an environmental factor such as temperature, ionic strength or partial pressure of gasses such as, for example, oxygen, carbon dioxide or carbon monoxide. For example, the cell(s) in the first state can be treated with a cellular stressor and the cell(s) in the second state not be treated with a cellular stressor, be treated with a cellular stressor different from the cellular stressor used to treat the cell(s) in the first state, or be treated with the cellular stressor used to treat the cell(s) in the first state but at a different concentration.

The determining step preferably includes determining the mRNA or protein in the cell(s) in the first state or the cell(s) in the second state, preferably both, using methods known in the art or later developed, such as nucleic acid hybridization

methods, nucleic acid arrays, immunoassays or peptidometrics. The identifying step preferably includes comparing the mRNA or protein in the cell(s) in the first state and the cell(s) in the second state. Such comparing can utilize automation and be computer assisted using, for example, pattern recognition or data mining (United States Patent No. 5,138,695 to Means et al., issued August 11, 1992; United States Patent No. 5,325,298 to Gallant, issued June 28, 1994; United States Patent No. 5,398,300 to Levey, issued March 14, 1995; United States Patent No. 5,471,627 to Means et al., issued November 28, 1995; United States Patent No. 5,619,709 to Caid et al., issued April 8, 1997; United States Patent No. 5,745,654 to Titan, issued April 28, 1998; United States Patent No. 5,687,306 to Blank, issued November 11, 1997; United States Patent No. 5,577,179 to Blank, issued November 19, 1996; United States Patent No. 5,469,536 to Blank, issued November 21, 1995 and United States Patent No. 5,345,313 to Blank, issued September 6, 1994).

II. Identification of Differentially Expressed Genes in Manipulated Cells

In another embodiment of the invention, differentially expressed organellar genes are identified in manipulated cells. Such cells include, but are not limited to (i) cybrid cells, *i.e.*, cell lines having a commonly derived nuclear component that has, in the case of a particular cybrid, been combined with a distinct cytoplasmic (mitochondria and/or chloroplast containing) component; (ii) rho^0 cells, *i.e.*, cells in which the amount of DNA in an organellar genome has been reduced or eliminated; and (iii) cells in which the wildtype genomic DNA (nuclear and/or organellar) has been mutated, added to or otherwise altered.

This aspect of the invention includes a method for identifying differentially expressed organellar genes in manipulated cells, including: providing one that is not a manipulated cell, providing at least one second cell that is a manipulated cell, determining the expression of genes in the first cell and the second cell, and identifying genes that are differentially expressed in the first cell(s) and the second cell(s). Preferably, the manipulated cell is a cybrid cell and the cell that is not a

manipulated cell is a parent cell of the manipulated cell, but this need not be the case. The first cell(s) and the second cell(s) can be provided in the same or different states.

Preferably, methods of the present invention use normal cells and cybrid cells (such as 1685) for a particular disease state, such as diabetes or Alzheimer's disease, to identify genes or proteins that are differentially expressed in the particular disease state. Optionally, the nucleic acid molecules and proteins identified by the methods of the present invention can be used to investigate cells, samples or tissues from normal and diseased states. In this aspect of the present invention, nucleic acid molecules identified by the present invention are used to interrogate cDNA libraries made from cells, samples or tissues that are appropriate for a particular disease state using, for example, nucleic hybridization methods. For example, for diabetes, tissue samples from skeletal muscle would be preferable, and for Alzheimer's disease, samples from the central nervous system, such as the brain, spinal column or fluids (preferably as soon after death as possible is the samples are taken post-mortem). The presence, absence, increased amount or decreased amount of a nucleic acid molecule identified by the present invention in cDNA libraries make from cells, samples or tissues of a diseased state as compared to cDNA libraries made using similar cells, samples or tissues of a non-diseased state indicates an association of that nucleic acid molecule, or the protein encoded by that nucleic acid molecule, with the disease state investigated. Optionally, a protein identified by the methods of the present invention can be measured in such samples using established methods, such as immunoassays or two-dimensional gel electrophoresis. The presence, absence, increased amount or decreased amount of a protein identified by the present invention in cells, samples or tissues of a diseased state as compared to cells, samples or tissues of a non-diseased state indicates an association of that protein, with the disease state investigated.

III. Identification of Differentially Expressed Genes during Cell Death

Another aspect of the invention involves the identification of nucleic acids that are differentially expressed during apoptosis (a.k.a. PCD, programmed cell death) and necrosis. Mutations and other alterations that limit a cell's response to

apoptosis may be events that occur during oncogenesis; that is, some cancer cells may represent the progeny of cells that have escaped apoptosis (Evan and Littlewood, *Science 281*:1317-1322, 1998). Nucleic acids that are differentially expressed during apoptosis, or biochemical events associated with apoptosis, can be used as probes in diagnostic, prognostic and pharmacogenomic assays useful in the therapeutic management of such diseases and disorders. Such nucleic acids can also be used to produce gene products that can be used as novel targets in methods for identifying pro-apoptotic agents useful to treat hyperproliferative diseases and disorders, as well as anti-apoptotic agents that can be used to treat, *e.g.*, degenerative diseases and disorders that are known to have or suspected of having an apoptotic component, including by way of non-limiting example, neurodegenerative diseases and disorders such as Alzheimer's disease and stroke (Barinaga, *Science 281*:1302-1304, 1998).

This aspect of the invention preferably includes a method for identifying nucleic acids that are differentially expressed during apoptosis, including: providing at least one first cell that is not apoptotic providing at least one second cell that is apoptotic state, determining the expression of genes in the first cell and the second cell, and identifying genes that are differentially expressed in the first cell and the second cell. An apoptotic cell is a cell that is expressing at least one gene, gene product or protein that can lead to apoptosis or have cellular conditions, such as redox potential or concentrations of ions or proteins in the cytosol or within or on an organelle, that can lead to apoptosis. The at least one first cell and the at least one second cell can also be provided in the same or different states.

In this embodiment of the invention, differentially expressed nucleic acids are identified in cells that have been induced to undergo apoptosis, or apoptotis-related processes, relative to cells that have not been so treated. Compounds generally known as apoptogens may induce apoptosis. Some apoptogens act only on cells having specific receptors; these include, as non-limiting examples, Tumor Necrosis Factor (TNF), FasL, NMDA, corticosterone and the like. However, many apoptogens do not require specific receptors, including by way of example and not limitation, herbimycin A, paraquat, ethylene glycols, protein kinase inhibitors (such as, *e.g.*, staurosporine,

calphostin C and caffeic acid phenethyl), chelerythrine chloride, Genistein, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine, Quercitin, N--[2-((*p*-bromocinnamyl)amino)ethyl]-5-5-isoquinolinesulfonamide, KN-93, d-*erythro*-sphingosine derivatives, MAP kinase inducers (such as, *e.g.*, anisomycin and anandamine), cell cycle blockers (such as, *e.g.*, aphidicolin, colcemid, 5-fluorouracil and homoharringtonine), acetylcholineesterase inhibitors (such as, *e.g.*, berberine), anti-estrogens (such as, *e.g.*, Tamoxifen), pro-oxidants (such as, *e.g.*, *tert*-butyl peroxide and hydrogen peroxide), free radicals (such as, *e.g.*, nitrous oxide), inorganic metal ions, such as, *e.g.*, Cadmium), DNA synthesis inhibitors (such as, *e.g.*, Actinomycin D, Bleomycin sulfate, Mitomycin C, camptothecin, daunorubicin, hydroxyurea, methotrexate and intercalators such as, *e.g.*, doxorubicin), protein synthesis inhibitors (such as, *e.g.*, cyclohexamide, puromycin and rapamycin), agents that affect microtubulin formation or stability (such as, *e.g.*, vinblastine, vincristine, colchicine, 4-hydroxyphenylretinamide and paclitaxel), and ionophores (such as, *e.g.*, ionomycin and valinomycin). Apoptosis may also be induced in some cell types by the withdrawal of growth factors such as, *e.g.*, interleukin-3 (IL-3). Furthermore, physical treatments, such as ultraviolet radiation, can induce apoptosis, as can intracellular bacteria such as *Staphylococcus aureus* (Bayles et al., *Infection and Immunity 66*:336-342, 1998).

IV. Identification of Genes that are Differentially Expressed in a Species-Specific Manner

Another aspect of the invention involves the identification of nucleic acids that are differentially expressed in a species-specific manner. By "species-specific manner" it is meant that nucleic acids encoding homologous gene products are up-regulated or down-regulated in a first organism belonging to one species but not in a second organism belonging to another species when cells from such species are exposed to a particular chemical compound or set of physical conditions. This embodiment of the invention is used in a variety of methods.

This aspect of the present invention includes a method for identifying nucleic acids that are differentially expressed in a species-specific manner, including:

providing one or more cells from a first species, providing one or more cells from a second species, determining the expression of genes in the cell(s) from the first species and the cell(s) from the second species and identifying genes that are differentially expressed in the cell(s) from the first species and the cell(s) from the second species. Preferably, the cell(s) from the first species and the cell(s) from the second species are cultured under the same or similar conditions, but that need not be the case. The cell(s) from the first species and the cell(s) from the second species can be provided in the same or different states.

For example, this embodiment of the invention can be used to identify homologous nucleic acids that are differentially expressed in a species-specific manner during apoptosis, and used to develop novel antibiotics. For example, species-specific nucleic acids of interest include without limitation homologs that are differentially expressed in apoptotic human cells relative to apoptotic cells from a eukaryotic pathogen or parasite, such as *e.g.*, trypanasomes (Ashkenazi and Dixit, 1998 *Science 281*:1305-1308) or insects. Such nucleic acids can be used to identify and produce gene products that can be used as novel targets in methods for identifying antibiotics that induce apoptosis in such pathogens and parasites but which do not induce apoptosis in the cells of their mammalian hosts. Alternatively, such nucleic acids can be used to identify and produce gene products that can be used as novel targets in methods for identifying compounds which protect mammalian cells from pro-apoptotic agents but which do not prevent or limit apoptosis in the cells of the eukaryotic pathogen or parasite. Such agents are expected to be useful for the prophylactic or therapeutic management of such pathogens and parasites.

In a related embodiment of the invention, nucleic acids that are differentially expressed in a species-specific manner include those that are up- or down-regulated during apoptosis in cells from undesirable plants (*e.g.*, weeds) but not in cells from desirable plants (*e.g.*, crops); or in cells from undesirable insects (in particular, members of the family *Lepidoptera* and other crop-damaging insects) but not in cells from desirable insects (*e.g.*, bees) or desirable plants. Such nucleic acids can be used to identify and produce gene products that can be used as novel targets in methods for

identifying herbicides and pesticides, respectively, that act by inducing apoptosis in such undesirable plants and insects but which do not induce apoptosis in the cells of desirable plants and insects. Alternatively, such nucleic acids can be used to identify and produce gene products that can be used as novel targets in methods for identifying compounds which protect cells from desirable plant and insect species from pro-apoptotic agents but which do not prevent or limit apoptosis in cells from undesirable plant and insect species exposed to such pro-apoptotic agents. Such agents are expected to be useful for the prophylactic or therapeutic management of such pathogens and parasites.

In a related aspect of this embodiment of the invention, the genomes of organelles of a desirable plant species are engineered to express a nucleic acid of interest that directs the production of a gene product which protects the cells of the desirable plant from herbicides (*e.g.*, paraquat) and insecticides that act by inducing apoptosis or by interfering with organellar functions (see, *e.g.*, Daniell et al., *Nature Biotechnology 16*:345-348, 1998). The nucleic acid that is introduced into the organellar genome may be one that is endogenous (*i.e.*, derived from the desirable plant) or one that is exogenous (derived from some other plant) in origin.

EXAMPLES

The following examples illustrate the invention and are not intended to limit the same. Those skilled in the art will recognize, or be able to ascertain through routine experimentation, numerous equivalents to the specific substances and procedures described herein. Such equivalents are considered to be within the scope of the present invention.

EXAMPLE 1

PREPARATION OF A CYBRID CELL LINE FOR DIFFERENTIAL GENE EXPRESSION EXPERIMENTS OF ALZHEIMER'S DISEASE

Gene expression in cybrid cells derived from a patient having Alzheimer's disease were compared to appropriate control cybrid cells. In particular, RNA species (or cDNA molecules derived therefrom) from the cybrid cell line

designated "1685 AD" were analyzed and compared to "MixCon" control cells. "MixCon" designates a Mixed Control composed of cybrids prepared using platelets from *n* normal patients (*n* = 2-3, depending on the particular experiment).

Procedures for preparing cybrid cells comprising mitochondria derived from patients having Alzheimer's disease have been previously described (Miller et al., *J. Neurochem. 67*:1897-1907, 1996; Swerdlow et al., *Neurology 49*:918-925, 1997; and U.S. patent application Serial No. 08/397,808, hereby incorporated by reference). The 1685 cybrid cell line is one example of a cybrid cell line of this type. The 1685 cybrid cell line was created by fusing platelets from an AD donor with SH-SY5Y neuroblastoma cells that had been made rho^0 by extended treatment with ethidium bromide.

To rule out the possibility of inadvertent transfection of donor nuclear DNA during cybrid formation (due to, e.g., the presence of white blood cells in the platelet preparation), ApoE genotyping was performed with DNA isolated from the AD donor, parental SH-SY5Y cells and AD cybrids by a primer extension assay that uses primers having the sequences 5'-GGCACGGCTGTCCAAGG (sense strand, SEQ ID NO:1) and 5'-CCCGGCCTGGTACACTG (antisense strand, SEQ ID NO:2). Various changes in the nucleotide sequence present in the ApoE gene between these two primers correspond to the ApoE1, ApoE2, ApoE3 and ApoE4 alleles (Mahley, *Science 240*:622-630, 1988). Primer extension using this primer pair thus interrogates a particular DNA sample for the presence or absence of these alleles (Livak and Haimer, *Hum. Mutat. 3*:379-385, 1994). Lymphocytes from the AD donor exhibited a heterozygous (ApoE3/ApoE4) allelic pattern. In contrast, the SH-SY5Y cells and 1685 cybrid cells displayed a homozygous (ApoE3/ApoE3) allelic pattern, thus indicating that the 1685 cybrid cells have the same nuclear complement as the parental SH-SY5Y cell line.

Mitochondrial DNAs from cell lines were also examined in order to confirm the transfer of the mitochondrial genome from the Alzheimer's patient. Total cellular DNA was prepared from a blood sample from the AD patient, rho^0 SH-SY5Y cells, parental SH-SY5Y cells, the 1685 AD cybrids and the MixCon cybrids. A multiplex primer extension assay was used to simultaneously interrogate mtDNA

positions 6366 and 6483 in PCR-generated fragments that encompass both loci (see pending U.S. patent application Serial No. 08/810,599, hereby incorporated by reference). In contrast to the parental SH-SY5Y and MixCon cybrids, total cellular DNA prepared from the 1685 cybrids and from a blood sample from the AD patient demonstrated a homoplasmic mutation at mtDNA position 6366 and the wildtype base at mtDNA position 6483.

In a typical differential gene expression experiment using cybrid cells, the following protocol was followed. MixCon and 1685 cybrid cells were thawed and cultured for approximately 2, 4 or 6 weeks. At the end of the culture period, the activities of two different components of the ETC (Complex I and Complex IV) in the cybrids was measured using the methods of Miller et al. (*J. Neurochem. 67*:1897-1907, 1996). These mitochondrial enzymes have been previously shown to be differentially active in AD platelets and in AD brains post mortem, and in cybrids in which the cytoplasmic component is derived from AD cells, in the following manner. Relative to control cybrids (i.e., those in which the cytoplasmic component is derived from normal, undiseased cells) Complex IV (cytochrome *c* oxidase, COX) activity is significantly decreased in AD cybrids, whereas Complex I (NADH:ubiquinone oxidoreductase) activity is not significantly different between the two (Davis et al., *Proc. Natl. Acad. Sci. USA 94*:4526-4531, 1997; Ghosh et al., "Mitochondrial Dysfunction and Alzheimer's Disease," Chapter 10 in: *Progress in Alzheimer's and Parkinson's Diseases*, Fisher et al., eds., Plenum Press, New York, 1998, pages 59-66; see also PCT application No. PCT/US95/04063, published as WO 95/26973, the entire contents of which are hereby incorporated by reference).

The activities of Complexes I and IV are monitored to ensure that the AD cybrids retain a phenotype associated with Alzheimer's disease. The results of a typical experiment are shown in Table 2. At the same time that samples were taken from the cybrids for the Complex I and IV assays, samples were also taken for preparation of total cellular RNA.

TABLE 2: Complex I and IV Activities in 1685 AD Cybrids

	MixCon			1685 AD Cybrids		
Days Out	Passage	Complex I Activity	Complex IV Activity	Passage	Complex I Activity	Complex IV Activity
23	107	23.0	2.00	106	35.5	1.41
37	108	33.5	1.84	107	23.6	1.47
58	112	28.8	2.23	112	33.3	1.18

EXAMPLE 2

PREPARATION OF RNA

In the present Example, RNA was prepared from MixCon cybrids and 1685 (AD) cybrids after 2, 4 and 6 weeks of culture. RNA was prepared from the cybrids using the TRIZOL® reagent (Life Technologies, Gaithersburg, MD; see U.S. Patent No. 5,346,994, hereby incorporated by reference) essentially according to the manufacturer's instructions. To remove DNA from the RNA preparations, samples were treated with RNase-free DNase I (Promega or Ambion) at a concentration of 1 to 5 u/uL for 20 to 30 minutes at 37°C.

EXAMPLE 3

REVERSE TRANSCRIPTION FOR DIFFERENTIAL DISPLAY

A. Design of Primers for Reverse Transcription

In order to generate DNA templates for amplification and analysis, it is necessary to reverse transcribe the RNA molecules in a sample. Of particular interest are those RNA molecules that encode polypeptides, known as messenger RNA (mRNA) molecules. In eukaryotic systems, nuclear mRNA molecules have a 5' poly(A^+) "tail" consisting of about 200 to 600 adenylic (A) residues that are added to the RNA molecule after transcription whereas, in the case of mitochondrial mRNAs, the 5' poly(A^+) "tail" is often somewhat shorter, i.e., about 50 to 60 adenylic residues. Either type of transcript is amenable to the procedure described below.

Reverse transcription and PCR amplification of subsets of the RNA molecules present in the samples was performed using the HIEROGLYPH™ mRNA Profile System (Genomyx Corp., Foster City, CA). The system is composed of five mRNA Profile Kits, each of which comprises 12 anchored oligonucleotide primers (AP-1, AP-2, etc.) in combination with 4 of 20 arbitrary 5' oligonucleotide primers (ARP-1, ARP-2, etc.).

Each anchored primer (AP) oligonucleotide has the sequence 5'-$(dT)_{10-12}$NM, where "NM" is, in each of the 12 AP oligonucleotides, GA, GC, GG, GT, CA, CC, CG, AA, AC, AG, AT or CT. Thus, each AP oligonucleotide is complementary to the 3' ends of some mRNA molecules, which have a poly(A^+) "tail." However, the identity of the "NM" nucleotides limits exact complementarity of a given AP oligonucleotide to a subset of the poly(A) RNA molecules in a sample. For example, an AP oligonucleotide having the sequence 5'-TTTTTTTTTTTTCG (SEQ ID NO:3) will have exact complementarity to only those mRNA molecules having the sequence 5'-CGAAAAAAAAAAAA (SEQ ID NO:4) at the beginning of their poly(A^+) "tail." Assuming that the identity of the two nucleotides immediately 5' from the first base of the poly(A^+) "tail" is random, each AP oligonucleotide will have exact complementarity to, and thus hybridize specifically to, 1 out of 12 (about 8%) of all of the mRNA species present in a sample.

B. Reverse Transcription

Regardless of which set of anchored primer (AP) oligonucleotides is or was employed, the RNA samples were combined with individual AP primer and heated (by incubation at 70°C for 5 minutes) and then chilled quickly on ice. Moloney murine leukemia virus (Mo-MLV or M-MLV) reverse transcriptase is used, in the presence of appropriate buffers and a combination of the 4 dNTPs necessary for DNA synthesis (i.e., dATP, dCTP, dGTP and dTTP), to carry out reverse transcription of the mRNA molecules according to protocols known in the art (*see, e.g.*, Dorit, "cDNA Amplification Using One-Sided (Anchored) PCR," Unit 15.6 in: *Short Protocols in Molecular Biology*, 2nd Ed., Ausubel et al., eds., John Wiley & Sons, New York, New

York, 1992, pages 15-21 to 15-27). More specifically, the reactions were carried out essentially according to the manufacturer's (Genomyx Corp.) instructions for first-strand cDNA synthesis reactions. Each reaction mix consisted of 20 uL (7.8 uL sterile nuclease-free H_2O; 4.0 uL 5x SuperScript II RT buffer; dNTP mix, 1:1:1:1, dATP:dTTP:dCTP:dGTP, 250 uM each; 100 mM DTT, 2.0 uL; and 0.2 uL of 200 Units/uL of SuperScript II RT enzyme). In the control –RT (no Reverse Transcriptase) reaction, 8.0 uL of sterile nuclease-free H_2O was added. Reactions were carried out in a thermal cycler with a heated lid and the following cycles were used: (I) 42°C for 5 minutes, (II) 50°C for 50 minutes, (III) 70°C for 15 minutes and (IV) hold at 4°C.

The products of the reverse transcription reactions are a group of DNA:RNA hybrid molecules, the DNA strand of each of which has a sequence that is the reverse complement of an mRNA molecule capable of specifically hybridizing to the specific AP oligonucleotide used in the particular instance. These reaction mixtures, referred to as "RT mixes," were stored at –20°C in a nonfrost-free freezer.

EXAMPLE 4

DIFFERENTIAL DISPLAY (DD) IN AD CYBRIDS

Following reverse transcription using the anchored primer, which produces a collection of RNA:DNA hybrid molecules, it was desirable to (a) prepare, amplify and label a set of the corresponding double-stranded cDNA molecules and (b) separate and evaluate the labeled double-stranded cDNA molecules. In the present instance, fluorescently labeled versions of the anchored and arbitrary primers were used in order to prepare labeled cDNA molecules, but it is also possible to labeled cDNA molecules by other means such as, e.g., labeling via radioactive isotopes. These reactions were carried out in duplicate in order to verify reproducibility.

Second-strand cDNA synthesis was primed using, in separate reactions, one of 20 arbitrary primers (e.g., M13r-ARP1, M13r-ARP2, etc. to M13r-ARP20; Genomyx Corp.). In each case, the arbitrary primer (ARP), corresponding to sense strand sequences located 5' from the poly-A tail of specific mRNA molecules, was hybridized to heat-denatured single-stranded (ss) DNA molecules. The reaction mixes

also contained labeled and unlabeled versions of the same anchored primer (AP) used in the reverse transcription reactions of the preceding Example. The fluorescent label used in the present Example was tetramethylrhodamine (TMR).

More specifically, each reaction mix contained 1.95 uL of sterile, nuclease-free H_2O; 1.0 uL of PCR Buffer II (without $MgCl_2$); 1.5 uL of 25 mM $MgCl_2$; 2.0 uL of dNTP mix, 1:1:1:1, dATP:dTTP:dCTP:dGTP, 250 uM each; 1.75 uL of 2 uM appropriate ARP primer (non-fluorescent version); 0.7 uL of fluorescent (TMR-labeled) version of 5 uM appropriate 3' AP primer (preceding reagents from Geonomyx Corp.); 1.0 uL of a specific "RT mix" (see preceding Example); and 0.1 uL of AmpliTaq® thermostable DNA polymerase (Perkin Elmer). The reaction mixes were incubated in a thermal cycler with a heated lid according to the following set of cycles: (I) 95°C for 2 minutes; (II) 4 cycles of 92°C for 15 seconds, 50°C for 30 seconds, and 72°C for 2 minutes; (III) 30 cycles 92°C for 15 seconds, 60°C for 30 seconds, and 72°C for 2 minutes; (IV) 72°C for 7 minutes; and (V) hold at 4°C. In general, caution was taken to avoid introducing nucleases into the reagents and the areas where the reactions were prepared and carried out, and aerosol-barrier, sterile, nuclease-free pipet tips were used. Each of the resultant "cDNA reactions" contains a set of fluorescently labeled PCR products corresponding to a particular subset of RNAs.

Four uL of each cDNA reaction was combined with 1.5 uL of fluoroDD loading dye in uncapped tubes. The DNAs were denatured and concentrated by heating the uncapped tubes at 95°C for 2 minutes in a thermal cycler with the lid open. The entire volume of the concentrated samples (about 2.5 to 3 uL) was loaded and electrophoresed on 5.6% polyacrylamide HR-1000™ clear denaturing gels (Genomyx). Gels containing the electrophoresed labeled PCR products were imaged using the genomyxSC scanner. Some representative results are shown in Figure 1.

Labeled PCR products from pairs of control and AD cybrid experiments were compared for bands of interest. Such bands include both (i) "up-regulated" genes, *i.e.*, bands that show an increased signal in the experimental (AD cybrid) lanes relative to the corresponding control (MixCon cybrid) lanes and (ii) "down-regulated" genes,

i.e., bands that show a decreased signal in the AD cybrid lanes relative to the corresponding control lanes.

Bands of interest were cloned in order to determine their nucleotide sequences (see following Example). Sequences were given "UNK" designations (*i.e.*, UNK1, UNK2, etc.; see Figures 5 through 32) until further characterized. In some instances, UNK sequences found to encode proteins of uncharacterized function were given "MG-UC" designations, and apparently novel UNK sequences were given "MG-NOV" designations.

As can be seen in Figure 1, both up-regulated and down-regulated nucleic acid species were identified in the AD cybrids in the present example. In particular, nucleic acids having the nucleotide sequences designated 1685 DD-Sequences #3 (UNK4, a.k.a. MG-UC2; SEQ ID NO:9), #5 (MG-NOV3; SEQ ID NO:11), and #6 (SEQ ID NO:____) showed decreased expression in the 1685 AD cybrids, as did UNK5, UNK10, UNK18 and UNK19 (SEQ ID NOS: 27, 32, 33, 44, and 45, respectively).

In contrast, nucleic acids having the nucleotide sequences designated 1685 DD-Sequences #1 (3-HICAH; SEQ ID NO:7), #2 (UNK3, a.k.a. MG-UC1; SEQ ID NO:8), and #4 (UNK2, a.k.a. MG-NOV2; SEQ ID NO:10), showed increased expression in the 1685 AD cybrids,), as did nucleic acids encoding SOD-1 (CuZnSOD; see below).

EXAMPLE 5

DETERMINATION OF NUCLEOTIDE SEQUENCES OF DIFFERENTIALLY DISPLAYED NUCLEIC ACIDS FROM AD CYBRIDS

The differentially expressed sequences of the preceding example were further characterized by determination of their nucleotide sequences. These sequences were determined as follows:

Labeled bands of interest (*i.e.*, either up- or down-regulated) were excised from gels by generating a digital image from the scanned gel and a virtual grid was used as an overlay to define the location of a band of interest. This location was

then transferred to a physical grid that was transferred to the actual gel. Gel fragments derived from the location of the band of interest were physically removed from the gel using a scalpel or similar instrument. DNA was eluted from the gel matrix by adding 50 uL of 10mM Tris to the excised gel fragments and incubation at 37°C for 30 to 60 minutes. One to 4 uL of the gel band eluent was subjected to further amplification in reaction mixes that further contained 19.4 to 16.4 uL, respectively, of sterile, nuclease-free H_2O (*i.e.*, the total volume of the gel band eluent and H_2O was 20.4 uL; 8.0 uL of Genomyx 5x Re-Amp Buffer; 3.2 uL of dNTP mix, 1:1:1:1, dATP:dTTP:dCTP:dGTP, 250 uM each; 4.0 uL of each primer (non-labeled versions of the pair of anchored and arbitrary primers used in the DD reactions were used); and 0.4 uL of 5 Units/uL AmpliTaq® thermostable DNA polymerase (Perkin Elmer). The reaction mixes were incubated in a thermal cycler with a heated lid according to the following set of cycles: (I) 95°C for 2 minutes; (II) 4 cycles of 92°C for 15 seconds, 60°C for 30 seconds, and 72°C for 2 minutes; (III) 25 cycles 92°C for 15 seconds, 60°C for 30 seconds, and 72°C for 2 minutes; (IV) 72°C for 7 minutes; and (V) hold at 4°C.

The resulting PCR products were cloned directly into linearized pCR2.1 vector DNA essentially according to the manufacturer's (Invitrogen, Carlsbad, CA) instructions using the "Original TA Cloning® Kit" (see http://www.invitrogen.com/manuals.html and U.S. Patent No. 5,487,993 for details). This linearized vector DNA is provided with single 3' deoxythymidine (dT) overhangs on each strand. Amplified DNA molecules produced by *Taq* polymerase have single 3' deoxyadenine (dA) residues and are thus complementary to, and can be ligated without further manipulation into, the linearized pCR2.1 DNA. (As will be appreciated by those skilled in the art, amplification products resulting from polymerases containing extensive 3' to 5' exonuclease activity, e.g., *Vent* and *Pfu* polymerases, lack such dA overhangs and would thus have to be further treated prior to ligation.)

Taq-amplified DNAs were combined with linearized pCR2.1 DNA and ligated using T4 DNA ligase and manufacturer (Invitrogen) supplied ligation buffer. The ligated DNAs were used to transform *Escherichia coli* cells. The *E. coli* strain used was XL1-Blue™ cells (Stratagene) having the phenotype *rec*A1 *end*A1 *gyr*A96 *thi*-1

*hsd*R17 *sup*E44 *rel*A1 *lac* [F' *pro*AB *lac*I^{q}ZΔM15 Tn*10* (Tet^R)]. Transformants were isolated as ampicillin-resistant colonies.

Strains MKN2 and MKN3, comprising pMKN2 (containing 1685 DD-Sequence #4, SEQ ID NO:10) and pMKN3 (containing 1685 DD-Sequence #5, SEQ ID NO:11), respectively, were deposited at the American Type Culture Collection (Manassas, VA) on March 4, 1999. Strain MKN2 was given the Accession No. 207149, and strain MKN3 was given the Accession No. 207150.

Plasmid DNA was isolated from transformants using the Wizard® Plus Series 9600 Miniprep Reagent System (Promega). The nucleotide sequences of the inserts in the isolated plasmid DNAs were determined in sequencing reactions that used primers that hybridize to regions present in the vector adjacent to the inserted DNAs [*i.e.*, a universal M13 reverse primer (5'-CAGGAAACAGCTATGAC, SEQ ID NO:5) and a T7 promoter primer (5'-TAATACGACTCACTATAGGG, SEQ ID NO:6), both from Invitrogen], and Prism® sequencing reagents (Perkin Elmer). Sequencing reaction products were purified by ethanol precipitation and then electrophoresed and analyzed using an ABI Prism 373A DNA Sequencer (Perkin Elmer) essentially according to the manufacturer's instructions. In some instances, the sequences of both the 5' and 3' ends of the insert were determined, resulting in sequences designated, for example, UNK10-5' and UNK10-3'.

The Sequence Navigator™ software (Perkin Elmer) was used for analysis of sequence data. Nucleotide sequences, and corresponding polypeptide sequences derived via *in silico* translation, were used to search the GenBank and Swissprot databases, respectively.

EXAMPLE 6

ANALYSIS OF NUCLEOTIDE SEQUENCES OF DIFFERENTIALLY DISPLAYED NUCLEIC ACIDS FROM AD CYBRIDS

A. Overlapping DD Sequences

As an initial matter, the UNK sequences were compared with each other in order to determine if any transcripts had been identified as differentially expressed in the cybrids more than once. This result is possible, as different pairs of primers used in differential display can result in PCR products that are of different length even though they are derived from the same transcript.

Several differentially displayed sequences were indeed found to overlap one another. In particular, UNK5 overlaps UNK10-5' and UNK10-3' (see Figure 33). In addition, UNK18 and UNK19 overlap one another (see Figure 34). These sequences are of particular interest as they indicate that the same transcript has been identified as differentially expressed in AD cybrids in two independent experiments, each of which uses a different set of PCR primers.

B. Types of Sequences and Homologies

In general, nucleotide sequences identified as being differentially displayed in the AD cybrids have been found to have nucleotide sequences that (1) are identical (or nearly so, reflecting sequence errors in the databases) to human nucleotide sequences present in the databases examined, (2) encode putative polypeptide sequences having some homology to the amino acid sequence of a known protein in humans and/or other species, and (3) have no apparent homology to any previously described nucleotide or polypeptide sequences (novel sequences). Sequences in classes (1) and (2) may be further characterized as being either (a) sequences encoding a gene product having characterized function(s) or (b) previously described sequences that encode a gene product whose function is unknown. In the present example, sequences of each type were identified by the preceding differential display (DD) methodology (Table 3).

TABLE 3: Differentially Expressed Genes in AD Cybrids as Determined by Differential Display (DD)

SEQ ID NO:	Gene Product Identity (if known)	Change in Expression in AD Cybrids
7	1685 DD-Sequence #1, a.k.a. 3-HICAH (3-hydroxyisobutyryl coenzyme A hydrolase)	Increased expression
8	1685 DD-Sequence #2, a.k.a. MG-UC1 (uncharacterized; corresponds to YAC377A1)	Increased expression
9	1685 DD-Sequence #3, a.k.a. MG-UC2 (UNK4) (corresponds to uncharacterized protein KIAA0711)	Decreased expression
10	1685 DD-Sequence #4, a.k.a. MG-NOV2 (UNK2) (unknown; novel sequence)	Increased expression
11	1685 DD-Sequence #5, a.k.a. MG-NOV3 (UNK3) (unknown; novel sequence)	Decreased expression

C. Previously Described Genetic Sequences

The sequences of interest in AD cybrids included nucleic acids encoding known gene products. Examples of such gene products included, but were not limited to, the following sequences:

1. UNK1 (1685 DD-Sequence #1; SEQ ID NO:7) was used to probe DNA databases and demonstrated a significant overlap with the cDNA for 3-hydroxyisobutyryl coenzyme A hydrolase (a.k.a. 3-HICAH; SEQ ID NO:7; see also Figure 2 and GenBank accession No. U66669).

2. SOD-1 (superoxide dismutase is an enzyme encoded by a cDNA (Accession No. X01662) having a sequence that overlaps an UNK sequence (SEQ ID NO:___; Figure 36). The DD results indicate that SOD-1 expression is decreased in AD cybrids.

3. UNK19 and UNK18 (SEQ ID NOS: 44 and 45, respectively; see also Figures 22, 23 and 34), which overlap and have increased expression in AD cybrids, were translated *in silico* in all six reading frames, and the resultant amino acid sequences were used to probe polypeptide and putative protein sequences. The search results yielded a number of matches to a reverse transcriptase homolog (designated

"ORF2" or "p150") found in long interspersed nuclear elements (LINEs). Many copies of LINEs are present in mammalian genomes; it is estimated that there are ~100,000 LINEs in the human genome, of which ~3,000 to ~4,000 are full-length. It has been reported that many LINEs are capable of retrotransposition (Sassaman et al., *Nature Genetics 16*:37-43, 1997), so these results may signify that, for whatever reason, LINEs are more likely to express p150, and thus retrotranspose, in AD cybrids. However, because many LINEs of nearly identical sequence are present in the genome, the present results do not allow one to distinguish between increased expression associated with one, as opposed to many LINEs. Accordingly, one possibility by way of non-limiting theory is that the increased expression of UNK19 and UNK18 may reflect the up-regulation of a single LINE, which may in turn result in the overexpression (*e.g.*, through trans-activation), or inappropriate expression, of genes located near that particular LINE.

D. Uncharacterized Genetic Sequences

Several previously described sequences of uncharacterized function were identified by the DD methodology.

1. MG-UC1 (a.k.a. UNK5, 1685 DD-Sequence #2, SEQ ID NO:8), which exhibited increased expression in AD cybrids, was used to probe databases for homologous and/or overlapping nucleotide sequences. A good match (E value = e-148) corresponds to sequences present on a cDNA encoding an uncharacterized protein designated "KIAA0711" (see Nagase et al., *DNA Res.* 5:277-286, 1998, and GenBank accession No. AB018254). When used to probe an EST database, SEQ ID NO:8 yielded many identical matches to several ESTs (Figure 38); this result indicates that MG-UC1 is expressed in a variety of tissues, including but not limited to, brain, testis, pineal gland, kidney, pancreas, liver, lung, *etc.*, in adult, as well as in fetal and infant tissues, in many instances.

The KIIA0711 putative protein has homology (E value = e-11 to e-10) to members of the family of proteins related to the Kelch protein of *Drosophila melanogaster*, which is a component of ring canals that regulates the flow of cytoplasm

between cells during oogenesis and other processes. However, another match of note (E value = 2e-10) occurs between KIIA0711 and the murine Keap1 protein. Keap1 represses the nuclear activation of antioxidant responsive elements by Nrf2 (Itoh et al., *Genes. Dev. 13*:76-86, 1999). Accordingly, by way of non-limiting theory, if the expression of Keap1 is increased in AD, the expected consequence would be that activation of antioxidant responsive elements would be decreased. This effect would work to increase the damage wrought by reactive oxygen species (ROS), where increased ROS production has been reported in AD cybrids and has been proposed as a possible contributing factor to neuronal death in AD (Swerdlow et al., *Neurology 49*:918-925, 1997).

2. MG-UC2 (a.k.a. UNK ____ , 1685 DD-Sequence #3, SEQ ID NO:9), the expression of which was decreased in AD cybrids, contains sequences corresponding to a bacterial artificial chromosome (BAC) clone known as BAC CIT987-SKA-237H1 that contains sequences from the p12 region of human chromosome 16 (see Figure 4 and GenBank accession No. AC002287). Like UNK19 and UNK18 (see above), the sequences in SEQ ID NO:9 are part of a set of repeated elements known as Alu elements, and, as a result, until further sequence information is obtained, one cannot be certain if the expression of a particular Alu element, or a gene associated with a particular Alu sequence, is increased in AD cybrids versus overexpression of two or more Alu elements and/or genes.

3. UNK5, UNK10-5' and UNK10-3' (SEQ ID NOS: 27 , 32 and 33, respectively) sequences overlap each other (Figure 33) and showed decreased expression in the AD cybrids. Although candidate homologs for UNK5 and UNK10 have been identified using other search strategies (see below), the following search strategy also yielded results. The nucleotide sequence "UNK5" (SEQ ID NO:27) was analyzed using the BLASTx program (Gish et al., *Nature Genetics 3*:266-272, 1993). This program translated, *in silico*, the UNK5 sequence in all six potential reading frames, and the resultant amino acid sequences were used to search for homologous amino acid sequences. The most homologous (E value = 4e-89) protein to UNK5-

encoded peptides is a putative polypeptide given the designation "AK000867" encoded by Accession No. dbj|BAA91401.1.

The AK000867 amino acid sequence was then used to probe polypeptide and putative amino acid sequences resulting from the *in silico* translation of nucleotide databases. The best-matching results were the uncharacterized putative protein "KIIA0138" (Accession No. gb|AAC14666.1) and scaffold attachment factor B ("Factor B"; Accession Nos. ref | NP 002958.1 and gb|AAC18697.1). Amino acid sequences from a conserved portion of the three polypeptide sequences were aligned (as shown in Figure 35) in order to generate the consensus sequence:

NIWVSGLSStTrAtDLKNLFsKYGKVvgAKVVTNARSPGArCYGfVTMStseE
atkCIaHLHrTELHGkmISVEKaKnEPagKKmSDkndeKSSkekssdvdr
(SEQ ID NO:63),

wherein upper case amino acid residues are absolutely conserved in all three amino acid sequences, and lower case amino acids represent the amino acid in two of the three sequences in most cases and the most neutral amino acid in those few positions where the three sequences each differed with respect to one another.

The amino acid consensus sequence was in turn used as a probe of peptide sequences in various databases. The search results (Figure 39) include a plethora of RNA-binding proteins, some of which are found in organelles (mitochondria or chloroplasts), one of which is a ribosomal protein. Thus, by way of non-limiting theory, the transcript from which UNK5, which is down-regulated in AD cybrids, ultimately derives from a gene encoding a protein that is likely to be a RNA-binding protein. This RNA-binding protein may be localized to an organelle, and may further be part of one or more ribonucleoprotein complexes, where such complexes include but are not limited to ribosomal subunits and ribosomes.

E. Novel Genetic Sequences

Several apparently novel sequences were identified in the DD screening described in this example. These are designated MG-NOV2 (a.k.a. UNK2; SEQ ID NO:10) and MG-NOV3 (SEQ ID NO:11). According to the DD results, MG-NOV2 expression is increased, whereas MG-NOV3 expression is decreased, in AD cybrids. Some of the sequences in MG-NOV2 (SEQ ID NO:10) are derived from *Alu* sequences, repetitive elements present in multiple copies in the human nuclear genome. SEQ ID NO:12 defines a non-repetitive portion of MG-NOV2 that can be used to specifically probe for nucleic acids or nucleotide sequences corresponding to MG-NOV2. Other apparently novel sequences include UNK4, UNK6, UNK7, UNK11, UNK12, UNK13, UNK16, UNK17, UNK20, UNK21-5', UNK21-3', UNK23, UNK24, UNK25-5', UNK25-3', UNK26-5', and UNK26-3'.

F. Further Analyses

In addition to the database searches for homology of differentially expressed sequences disclosed herein (*e.g.*, the various UNK sequences) to other nucleotide sequences, additional homology searches using different search strategies were carried out to help identify the function of the differentially displayed sequences. The results of these searches are shown in Figure 37. The figure indicates the results from the following search strategies:

"Genbank nt" indicates the results from searches using each UNK nucleotide sequence as a probe of the Genbank DNA database.

"Genbank nr" indicates the results from a search wherein each UNK nucleotide sequence was translated *in silico* in all 6 potential reading frames to yield peptide sequences that were compared to peptide sequences in various databases.

"Human EST" indicates the results from searches using each UNK nucleotide sequence as a probe of the Expressed Sequence Tag (EST) DNA database.

Because the EST database is generally considered to have relatively poor quality sequences, the Unigene database was also searched. This database assembles various EST sequences into virtual transcripts, a process that is believed to eliminate

many sequencing errors in the EST sequences. The results of these searches are given under the heading "Unigene".

In Figure 37, the degree of homology was calculated according to E values, which are presented therein. An "E value" (expectation value) is a result of a FASTA analysis that indicates the probability that a match between two sequences is due to random chance (Pearson et al., *Proc. Natl. Acad. Sci. U.S.A. 85*:2444-2448, 1988). E values are typically presented in exponential form (*i.e.*, "E-43" is an abbreviation for 1^{-43}). The closer the E value is to zero, the greater the likelihood that the homology between the sequences being compared is not due to random chance. For example, "E-50" is a smaller number than "E-10" and thus represents a better potential "match" between the sequences.

Some candidate homologies of note included, but were not limited to, those of UNK9 and UNK11 to neuronal thread protein (NTP), a protein that has been implicated in AD; UNK15 (both 3' and 5') to related tyrosine kinases; UNK16 (3' and 5') to DNA repair enzymes; UNK22-3' to mitochondrial uncoupling protein 2; and UNK11 and UNK12 to ribosomal proteins.

EXAMPLE 7

CONFIRMATION OF DIFFERENTIAL EXPRESSION IN AD HYBRIDS BY Q-RTPCR

In order to confirm the differential expression of a particular gene product, it is necessary to validate the results from a first method of monitoring differential expression (in this instance, the above-described differential display) via a second, independent method. In the present example, quantitative real-time polymerase chain reaction (Q-RTPCR) was used to validate the six sequences of interest identified in the preceding Example.

A. Reverse Transcription for Q-RTPCR

The RNA prepared from normal and AD cybrids according to Example 2 was used in reverse transcription reactions. First strand cDNA was synthesized with the SuperScript™ pre-amplification system (Life Technologies) using an oligo(dT) primer.

B. Design of Primers for Q-RTPCR

In the remainder of the Example, the RNA:DNA hybrid molecules produced by these reactions were used as templates in PCR amplification reactions using primers derived from the nucleotide sequences determined as in the preceding Example. The sequences of these oligonucleotide primers, designed to correspond to (reverse primers) or be complementary to (forward primers) sense strand sequences in the 3' region of the nucleotide sequences of interest, are described in Table 4.

TABLE 4 Sequences of Primers for Quantitative Real Time PCR (Q-RTPCR)

SEQ ID NO:	Oligonucleotide Sequence (5' → 3')	Template Nucleic Acid of Interest	Template Coordinates[1]
13	GGATTCAGACTAAAAGGAAGAGATGTG	3-HICAH[2]	40 → 66 (f)
14	AAATCTTCCTCTAACATGGCCAACT	3-HICAH[2]	131 → 107 (r)
15	CGCCAAGTGGATGGATTTG	MG-UC1[3]	12 → 30 (f)
16	GGAGGAGCTTTGATCTCACATGA	MG-UC1[3]	82 → 63 (r)
17	GATTCAGAGCTTGCCCTAGCA	MG-UC2[4]	96 → 116 (f)
18	CCAGTGTGAACCTTTTTCACTGTT	MG-UC2[4]	178 → 155 (r)
19	AGAAAATTTGTGAGACATCTTTGTGTAAA	MG-NOV2[5]	352 → 360 (f)
20	CTGGTTATAAGTTATATCCTCGCAGCTA	MG-NOV2[5]	432 → 405 (r)
21	GAGCTGATACTATTCCCACTGAAACTATT	MG-NOV3[6]	448 → 476 (f)
22	TGTCTCTACCAGGTTTTGGTATTAGGA	MG-NOV3[6]	550 → 524 (r)

Notes for Table 6:

[1] "f", forward; "r", reverse.

[2] SEQ ID NO:7, 1685 DD-Sequence #1, 3-hydroxyisobutyryl coenzyme A hydrolase.

[3] SEQ ID NO:8, 1685 DD-Sequence #2, Uncharacterized sequence MG-UC1, 3' region similar to YAC clone 377A1 and cDNA for uncharacterized protein KIAA0711.

[4] SEQ ID NO:9, 1685 DD-Sequence #3, Uncharacterized sequence MG-UC2, 3' region similar to BAC clone CIT987-SKA-237H1.

[5] SEQ ID NO:10, 1685 DD-Sequence #4. Novel sequence MK-NOV2.

[6] SEQ ID NO:11, 1685 DD-Sequence #5, Novel sequence MK-NOV3.

C. Confirmation of Primer Specificity

The Q-RTPCR analyses described in the present Example involve the quantification of amplified DNA based on the fluorescence of an intercalating dye, SYBR® Green (Perkin Elmer Applied Biosystems, Foster City, CA; see http://www2.perkinelmer.com/ab/techsupp/doclib/ pcr/protocols/pdf/SYBR_Green.pdf and U.S. Patent No. 4,304,886, hereby incorporated by reference). Because the SYBR® Green dye fluoresces to a greater degree when bound to any double-stranded (ds) DNA, it is necessary to perform an initial set of PCR reactions to confirm that the PCR primers of choice amplify a single DNA species.

PCR reactions were carried out using the primers described in Table 4 and the DNA templates produced by the reverse transcription reactions described in section A of this Example. The RNA:DNA molecules produced by reverse transcription were used as templates and the appropriate primers were added to reaction mixtures. Amplification was carried out using Taq DNA polymerase (Perkin Elmer) and the following cycles: (I) 95°C, 10 minutes; (II) 30 cycles of 95°C, 1 minute, 60°C, 1 minute, 72°C, 1 minute; (II) 72°C for 4 minutes; then (III) hold at 4°C.

The PCR products, and appropriate molecular size markers, were electrophoresed, stained with ethidium bromide and visualized via fluorescence. In each instance, a single band of the predicted molecular weight was detected, confirming that the primer pair amplifies a sequence corresponding to the specific nucleic acid of interest.

D. Quantitation of Nucleic Acids of Interest via Q-RTPCR

The use of real time PCR to quantitate levels of specific nucleic acids has been described in the art (Heid et al., *Genome Research 6*:986-994, 1996; Gibson et al., *Genome Research 6*:995-1001, 1996; see Freeman et al., *BioTechniques 26*:112-125, 1999, for a recent review; all references being hereby incorporated by reference). For ease of understanding, a brief explanation of quantitative real time PCR (Q-RTPCR) follows.

Until recently, the traditional means of measuring the products of a specific PCR reaction was the "end-point" method of analysis, in which the reaction products are measured and quantitated after the amplification reactions are completed. In contrast, "real-time" PCR monitors amplification reactions in the thermal cycler as they progress. Q-RTPCR provides for improved quantification, because quantification is achieved most accurately during the linear range of amplification, and more information about the amplification reactions is obtained for each cycle. For example, the normalized (*i.e.*, to a passive reference dye that does not bind DNA) fluorescence intensity ("ΔR_n"), which indicates the magnitude of the signal generated by a given set of PCR conditions, can be measured during each cycle.

From such data, the cycle at which a statistically significant increase in ΔR_n is first detected can be determined. The "threshold cycle" or "C_T value" is determined at one log above the signal first detected and provides a quantitative measure of the amount of the input nucleic acid template of interest present in the original sample.

In order to correct for sample-to-sample variation, an internal RNA normalizer is used in Q-RTPCR. The RNA normalizer may be an endogenous RNA species, for example, an mRNA encoding a constitutively-expressed protein like actin or glyceraldehyde-3-phosphate dehydrogenase (GAPDH), or a ribosomal RNA such as 18S or 28S rRNA; RNA molecules produced *in vitro* may also be used as normalizers. Results of Q-RTPCR analyses are thus often expressed as relative amounts.

For instance, when the normalizer is actin and the nucleic acid that is being quantitated is 3-hydroxyisobutyryl coenzyme A hydrolase (3-HICAH; SEQ ID NO:7), the relative amount of 3-HICAH RNA in a sample is determined as compared to the normalizer actin according to standard curves created for both gene sequences for each RNA sample (*i.e.*, AD and control). Standard curves were typically prepared using 4 to 5 different amounts of input RNA in triplicate reactions. For example, the following amounts of input RNA might be evaluated in triplicate: (I) 0.1 ng, 0.5 ng, 1 ng and 5 ng or (II) 0.3 ng, 1 ng, 3 ng and 10 ng). Standard curves were plotted as log

input ng (x axis) versus Ct (y axis, also log scale). For each standard curve, the slope (m) and the y-intercept (b) were calculated using standard analysis software.

The log input amount for the normalizer (nN) is calculated for a given Ct (Ct^0). For example, when $Ct^0 = 20$,

$$nN = \frac{(20-b_N)}{m_N}$$

For a specific target (T) sequence of interest, Ct^T (the Ct required to reach a log input amount equal to nN) is determined by the formula:

$$Ct^T = (m_T \times nN) + b_T$$

The normalized target Ct (normalized Ct^T) is calculated according to the formula:

$$\text{normalized } Ct^T = Ct^T - Ct^0$$

The Change in Expression, *i.e.*, the comparative ratio of the target sequence of interest in AD (1685) versus control (MixCon) cybrids is calculated according to the formula:

$$\text{Change in Expression} = 2^{(\text{Control normalized } Ct^T) - (\text{AD normalized } Ct^T)}$$

In the present Example, PCR reactions were performed using *Taq* DNA polymerase and the primers described in Table 6 with the following cycles: (I) 50°C for 2 minutes, 95°C for 10 minutes; (II) 40 cycles of 95°C for 15 minutes, 60°C for 1 minute; and then (III) cooling to room temperature. PCR products were detected with SYBR® Green detection reagents (Perkin Elmer) using the ABI Prism 7700 Sequence Detection System (Perkin Elmer).

The relative (normalized) amounts of each candidate gene of interest (a.k.a. DD-Sequences #1 to #5) compared to the normalizer gene (actin) were calculated according to the preceding formulae. Comparative ratios of [the normalized amount of DD-Sequence in the 1685 AD cybrids] to [the normalized amount of DD-Sequence in MixCon control cybrids] were calculated for each DD-Sequence. The results are shown in Table 5.

TABLE 5: Differentially Expressed Genes in AD Cybrids as Determined by Differential Display (DD) and Quantitative Real Time PCR (Q-RTPCR)

SEQ ID NO:	Gene Product	Change in Expression (AD vs. control): DD	Change in Expression (AD vs. control): Q-RTPCR
7	3-HICAH	↑	↑ 2.2x
8	MG-UC1	↑	↑ 1.9x
9	MG-UC2	↓	↓ 2.5x
10	MG-NOV2	↑	↑ 3.3x
11	MG-NOV3	↓	

These results confirmed the differential expression of RNAs having sequences corresponding to 3-HICAH (SEQ ID NO:7). MG-UC1 (SEQ ID NO:8), MG-UC2 (SEQ ID NO:9) and MG-NOV2 (SEQ ID NO:10). and these sequences are thus derived from *bona fide* differentially expressed genes in AD cybrids. The gene products corresponding to these sequences are therefore implicated in Alzheimer's disease and may be used to develop diagnostic, prognostic and therapeutic compositions and methods.

For the accompanying SEQUENCE LISTING, the indicated summary comments for the indicated SEQ ID NOs. are provided:

SEQ ID NO	Summary Comments
1	Forward PCR primer for ApoE genotyping
2	Reverse PCR primer for ApoE genotyping

SEQ ID NO	Summary Comments
3	None
4	None
5	M13 reverse primer
6	T7 Promoter primer
7	1685 DD-Sequence #1 3-hydroxyisobutyryl coenzyme A hydrolase
8	1685 DD-Sequence #2 Uncharacterized sequence MG-UC1 3' region similar to YAC clone 377A1and to cDNA for uncharacterized protein KIAA0711
9	1685 DD-Sequence #3 Uncharacterized sequence MG-UC2 3' region similar to BAC clone CIT987-SKA-237H1
10	1685 DD-Sequence #4 Novel sequence MG-NOV2
11	1685 DD-Sequence #5 Novel sequence MG-NOV3
12	Non-repetitive portion of 1685 DD-sequence #5 Novel sequence MG-NOV2
13	Forward primer for Q-RTPCR For 1685 DD-Sequence #1 3-hydroxyisobutyryl coenzyme A hydrolase
14	3-HICAH reverse primer for Q-RTPCR For 1685 DD-Sequence #1 3-hydroxyisobutyryl coenzyme A hydrolase
15	Forward primer for Q-RTPCR For 1685 DD-Sequence #2 3' region similar to YAC clone 377A1

SEQ ID NO	Summary Comments
16	Reverse primer for Q-RTPCR For 1685 DD-Sequence #2 Uncharacterized sequence MG-UC1 3' region similar to YAC clone 377A1
17	Forward primer for Q-RTPCR For 1685 DD-Sequence #3 Uncharacterized sequence MG-UC2 3' regions similar to BAC clone 987-SKA-237H1
18	Reverse primer for Q-RTPCR For 1685 DD-Sequence #3, Uncharacterized sequence MG-UC2 3' region similar to BAC clone CIT987-SKA-237H1
19	Forward primer for Q-RTPCR For 1685 DD-Sequence #4 Novel sequence MG-NOV2
20	Reverse primer for Q-RTPCR For 1685 DD-Sequence #4 Novel sequence MG-NOV2
21	Forward primer for Q-RTPCR For 1685 DD-Sequence #5 Novel sequence MG-NOV3
22	Reverse primer for Q-RTPCR For 1685 DD-Sequence #5 Novel sequence MG-NOV3

From the foregoing, it will be appreciated that although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. All publications, including patent documents and scientific articles, referred to in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication were individually incorporated by

reference. All headings are for the convenience of the reader and should not be used to limit the meaning of the text that follows the heading, unless so specified.

CLAIMS

What is claimed is:

1. A method for identifying a factor encoded by a gene that is differentially expressed, comprising:

comparing (i) expression of a plurality of genes in at least one first cell that is in a first state to (ii) expression of a plurality of genes in at least one second cell that is in a second state, thereby identifying a gene that is differentially expressed in said first state relative to said second state, and therefrom identifying a factor encoded by a gene that is differentially expressed.

2. The method of claim 1 wherein the first cell is a manipulated cell.

3. The method of claim 1 wherein the second cell is a manipulated cell.

4. The method of either claim 2 or claim 3 wherein the manipulated cell is a cybrid cell.

5. The method of either claim 2 or claim 3 wherein the manipulated cell is a ρ^0 cell.

6. The method of claim 1 wherein the first cell is a manipulated cell and the second cell is a manipulated cell.

7. The method of claim 6 wherein at least one of said first and second cells is a cybrid cell.

8. The method of claim 6 wherein both of said first and second cells are cybrid cells.

9. The method of claim 6 wherein at least one of said first and second cells is a ρ^0 cell.

10. The method of claim 6 wherein both of said first and second cells are ρ^0 cells.

11. The method of claim 1 wherein the factor is an organellar factor.

12. The method of claim 11 wherein the organellar factor is protein.

13. The method of claim 11 wherein the organellar factor is a nucleic acid.

14. The method of claim 11 wherein the factor is differentially expressed in an organelle associated disease.

15. The method of claim 11 wherein the factor is differentially expressed in response to treatment with an agent that alters at least one organellar function.

16. The method of claim 15 wherein the organellar function is a mitochondrial function.

17. The method of claim 16 wherein the mitochondrial function is selected from the group consisting of electron transport chain activity, oxidative phosphorylation, ATP production, intracellular calcium homeostasis, apoptosis, mitochondrial permeability transition and free radical production.

18. The method of claim 11 wherein the factor is differentially expressed in response to treatment with an agent selected from the group consisting of a stressor and an apoptogen.

19. The method of claim 11 wherein the factor is differentially expressed in a species specific fashion.

20. The method of claim 1 wherein the first state and the second state are different and at least one of said first and second states is a disease state.

21. The method of claim 20 wherein the disease is an organelle associated disease.

22. The method of claim 1 wherein the first state and the second state are different and at least one of said first and second states is a response to a stressor.

23. The method of claim 22 wherein the stressor is a molecule.

24. The method of claim 22 wherein the stressor is an environmental factor.

25. The method of claim 1 wherein the step of comparing comprises determining mRNA in each of said first and second cells.

26. The method of claim 1 wherein the step of comparing comprises determining protein in each of said first and second cells.

27. The method of claim 1 wherein said first and second cells are derived from the same clone.

28. The method of claim 1 wherein said first and second cells are derived from different species.

29. The method of claim 1 wherein the first state and the second state are different and at least one of said first and second states is selected from the group consisting

of a metabolic state, a respiratory state, a cell cycle state, a pathologic state, a differentiative state, a maturational state, a genetic state, an apoptotic state, an excitotoxic state and a pharmacological state.

30. A method of diagnosing a disease comprising contacting a biological sample from an individual suspected of having said disease with at least one factor identified according to the method of claim 1.

31. The method of claim 30 wherein the factor is a nucleic acid.

32. The method of claim 31 wherein the nucleic acid has a sequence selected from the group consisting of:

(a) SEQ ID NOS:8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;

(b) the reverse complements of SEQ ID NOS:8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22; and

(c) equivalents thereof.

33. A method of diagnosing a disease comprising contacting a biological sample from an individual suspected of having said disease with an antibody that specifically binds a factor identified according to the method of claim 1.

34. The method of claim 33 wherein the factor is a protein.

35. A cell line selected from the group consisting of cybrid cell line 1685, ATCC 207149 and ATCC 207150.

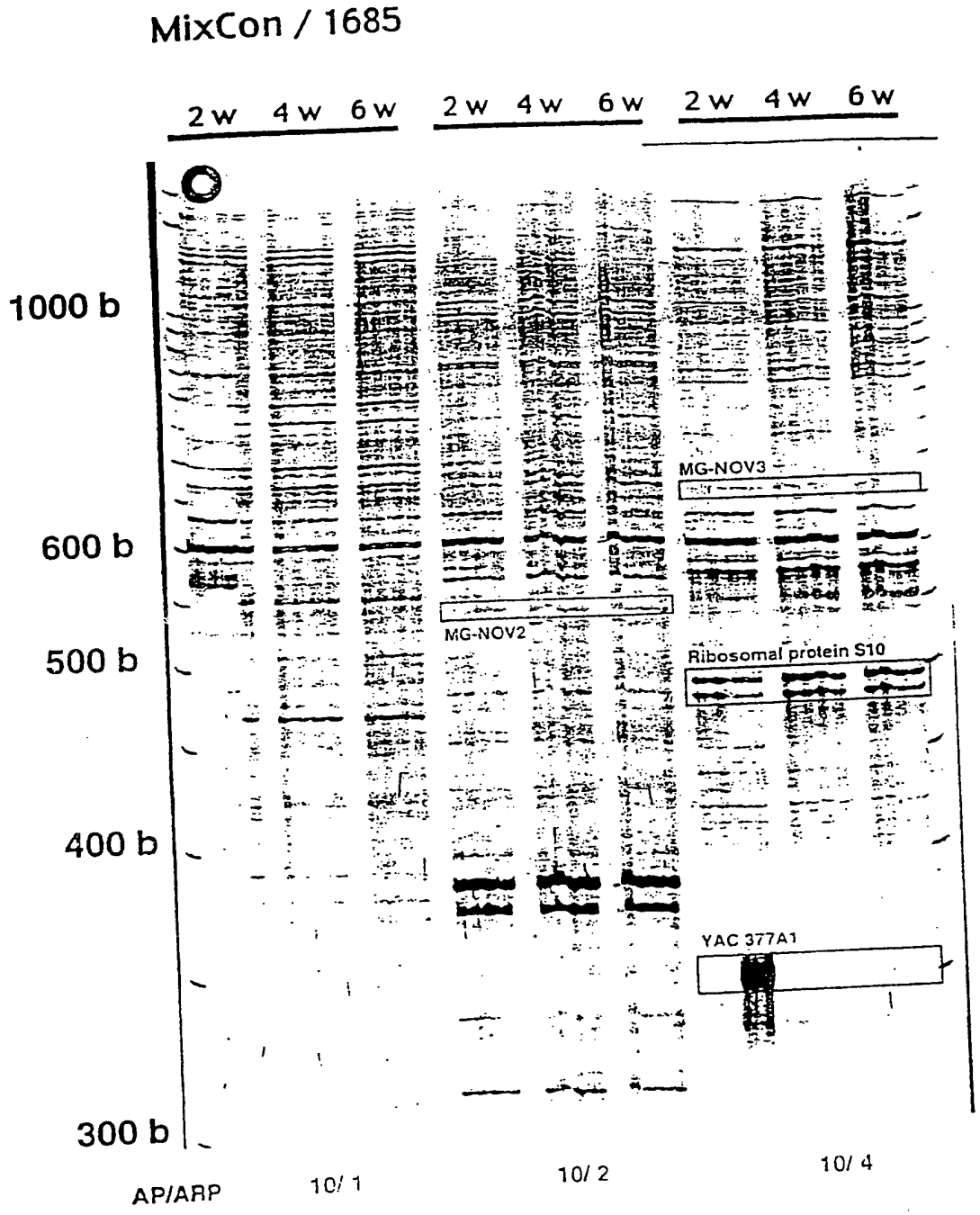
MixCon / 1685
2w 4w 6w 2w 4w 6w 2w 4w 6w
1000 b
600 b
500 b
400 b
300 b
MG-NOV3
MG-NOV2
Ribosomal protein S10
YAC 377A1
AP/ARP
10/ 1
10/ 2
10/ 4

Figure 1

```
QUERY:      1685 DD-Sequence #1 (SEQ ID NO:7)

SBJCT:      gb|U66669|HSU66669 Homo sapiens 3-hydroxyisobutyryl-coenzyme A hydrolase
            mRNA, complete cds (Length = 1311)

Strand = Plus / Plus

QUERY: 1    CGACTCCAAGGAAAACTTGGTTACTTCCTTGCATTAACAGGATTCAGACTAAAAGGAAGA 60
            ||||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||
SBJCT: 605  CGACTCCAAGGAAAACTTGGTTACTTCCTTGCATTAAC-GGATTCAGACTAAAAGGAAGA 663

QUERY: 61   GATGTGTACAGAGCAGGAATTGCTACACACTTTGTATATTCTGAAAAGTTGGCCATGTTA 120
            ||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||
SBJCT: 664  GATGTGTACAGAGCAGGAATTGCTACACACTTTGTAGATTCTGAAAAGTTGGCCATGTTA 723

QUERY: 121  GAGGAAGATTTGTTAGCCTTGAAATCTCCTTCAAAAGAAAATATTGCATCTGTCTTAGAA 180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
SBJCT: 724  GAGGAAGATTTGTTAGCCTTGAAATCTCCTTCAAAAGAAAATATTGCATCTGTCTTAGAA 783

QUERY: 181  AATTACCATACAGAGTCTAAGATTGATCGAGACAAGTCTTTTATACTTGAGGAACACATG 240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
SBJCT: 784  AATTACCATACAGAGTCTAAGATTGATCGAGACAAGTCTTTTATACTTGAGGAACACATG 843

QUERY: 241  GACAAAATAAACAGTTGTTTTTCAGCCAATACTGTGGAAAGAAATTATTGAAAACTTACA 300
            |||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||
SBJCT: 844  GACAAAATAAACAGTTGTTTTTCAGCCAATACTGTGG-AAGAAATTATTGAAAACTTACA 902

QUERY: 301  GCAAGATGGTTCATCTTTTTGCCCCTAGAACAATTGAAGGTAATTAATAAA-TGTTCTCC 359
            |||||||||||||||||| | ||||||| |||||||||||||||||||| ||||||
SBJCT: 903  GCAAGATGGTTCATCTTTT-G-CCCTAGAGCAATTGAAGGTAATTAATAAAATGTCTC-- 958

QUERY: 360  CAACATCTCTTAAAGATCCACCCTAAGGCCC                              390
            |||||||||| |||||||| || |||||||
SBJCT: 961  CAACATCTCT-AAAGATC-ACACTAAGGCAA                              989
```

Figure 2

QUERY: 1685 DD-Sequence #2 (SEQ ID NO:8)

SBJCT1: gb|AF009203|AF009203 Homo sapiens YAC clone 377A1 unknown mRNA, 3'untranslated region (Length = 2167)

SBJCT2: dbj|AB018254|AB018254 Homo sapiens mRNA for KIAA0711 protein, complete cds (Length = 6706)

Strand = Plus / Plus / Plus

```
QUERY: 0001                                                                  GCTAG 0005

SBJCT1:1804 GGAAGCCTGGACTGTGCAGCCTTCGGGCACCCGGCACAGACACTGTGCTGGCAGGAGCTT 1863
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
SBJCT2:6350 GGAAGCCTGGACTGTGCAGCCTTCGGGCACCCGGCACAGACACTGTGCTGGCAGGAGCTT 6409

QUERY: 0006 CAGACACGCCAAGTGGATGGATTTGGATTGAACGCATATGAAACAGGAGACGGGTTCTCA 0065
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
SBJCT1:1864 CAGACACGCCAAGTGGATGGATTTGGATTGAACGCATATGAAACAGGAGACGGGTTCTCA 1923
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
SBJCT2:6410 CAGACACGCCAAGTGGATGGATTTGGATTGAACGCATATGAAACAGGAGACGGGTTCTCA 6469

QUERY: 0066 TGTGAGATCAAAGCTCCTCCAAAGCCTGTTCAAGCTCTAAGCGATTCTCAAATGTTACCA 0125
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
SBJCT1:1924 TGTGAGATCAAAGCTCCTCCAAAGCCTGTTCAAGCTCTAAGCGATTCTCAAATGTTACCA 1983
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
SBJCT2:6470 TGTGAGATCAAAGCTCCTCCAAAGCCTGTTCAAGCTCTAAGCGATTCTCAAATGTTACCA 6529

QUERY: 0126 TTTATTAAAGGTAAACTACACCTGTTGAAGGCCAAGTTCAGGGCAGCTGTTGTGATCTGT 0185
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
SBJCT1:1984 TTTATTAAAGGTAAACTACACCTGTTGAAGGCCAAGTTCAGGGCAGCTGTTGTGATCTGT 2043
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
SBJCT2:6530 TTTATTAAAGGTAAACTACACCTGTTGAAGGCCAAGTTCAGGGCAGCTGTTGTGATCTGT 6589

QUERY: 0186 GTAGTTAATGTATTTATTAATGCTTGACTTTTAAAATCCTGGGCATAAATAGTGCAGAGC 0245
            |||||||||||||||||||||||||||||||||||| ||||||||||||||||||||
SBJCT1:2044 GTAGTTAATGTATTTATTAATGCTTGACTTTTAAAAYCCTGGGCATAAATAGTGCAGAGC 2103
            |||||||||||||||||||||||||||||||||||| ||||||||||||||||||||
SBJCT2:6590 GTAGTTAATGTATTTATTAATGCTTGACTTTTAAAATCCTGGGCATAAATAGTGCAGAGC 6649

QUERY: 0246 CTCGTATGTTTGTCAGCTCATGCCGAGATGAAATAAATCACGCAGAAAGTGCCAGTCCTA 0305
            |||||||||||||||| ||||||||||||||||||||||||||||||||||||||  |
SBJCT1:2104 CTCGTATGTTTGTCAGTTCATGCCGAGATGAAATAAATCACGCAGAAAGTGCCAGTC--A 2161
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
SBJCT2:6650 CTCGTATGTTTGTCAGTTCATGCCGAGATGAAATAAATCACGCAGAAAGTGCCAGTC    6706

                                                                             0314
QUERY: 0306 AAAAAAAAA
            ||||||
SBJCT1:2162 AAAAAA                                                           2167
```

Figure 3

```
QUERY:      1685 DD-Sequence #3 (SEQ ID NO:9)

SBJCT:      gb|AC002287|HUAC002287 Homo sapiens Chromosome 16p12 BAC clone CIT987-
            SKA-237H1 (genomic sequences; Length = 188636)

Strand = Plus / Minus

QUERY: 000001 GACCATTGCATCCTACTACATCTGCATTCCACTCAGCAGGAAGAGGGTGTAGAAATAAAT 000060
              |||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
SBJCT: 122700 GACCCTTGCATCCTACTACATCTGCATTCCACTCAGCAGGAAGAGGGTGTAGAAATAAAT 122641

QUERY: 000061 GAAGACTATCCAAGAGAGAGCAAGCAGAGGTCATTGATTCAGAGCTTGCCCTAGCAAAGA 000120
              |||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
SBJCT: 122640 GAAGACTATCCAAAAGAGAGCAAGCAGAGGTCATTGATTCAGAGCTTGCCCTAGCAAAGA 122581

QUERY: 000121 GTCTTGCATTTGGCAGAAACTCACAGGCTGGCAGAACAGTGAAAAAGGTTCACACTGGAA 000180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
SBJCT: 122580 GTCTTGCATTTGGCAGAAACTCACAGGCTGGCAGAACAGTGAAAAAGGTTCACACTGGAA 122521

QUERY: 000181 AAGAGAGAAGGCTTCAGGGGTGCCTGATTGGAGGTAGTTGGCGTANGAAAGCTGGAAGTG 000240
              |||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||
SBJCT: 122520 AAGAGAGAAGGCTTCAGGGGTGCCTGATTGGAGGTAGTTGGCGTAGGAAAGCTGGAAGTG 122461

QUERY: 000241 GGCTCATTANAAGTGGGGCATCCGGCTGGGTGCAGCAGCTCACACCTATAATCCCAGCAC 000300
              ||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||
SBJCT: 122460 GGCTCATTAGAAGTGGGGCATCCGGCTGGGTGCAGCAGCTCACACCTATAATCCCAGCAC 122401


QUERY: 000301 TTTGGGAGGCTAAGGCTGGCAGATCCCTTGAGCCTAGGAGTGCGAGACCAGCCTGGGCAA 000360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
SBJCT: 122400 TTTGGGAGGCTAAGGCTGGCAGATCCCTTGAGCCTAGGAGTGCGAGACCAGCCTGGGCAA 122341


QUERY: 000361 CATGGCAAAACCCTGTCTCTATGAAAAAAAAAAAA                          000395
              |||||||||||||||||||||||||||||||| ||
SBJCT: 122340 CATGGCAAAACCCTGTCTCTATGAAAAAAAAACAAAAGAAAAGAAAAAATAGCTGGGCAT 122281
```

Figure 4

Figure 5

UNK1: 394 nt
CGACTCCAAGATAGGCAGATTGTGGAGAAATAAATATTTCCCTAGTCATTGTGATTACATTCCTAATGGAC
CTTCTCTGGTGCTGATACTGAAATAGTACAAAAAGTTGTCAGTACCTTTCAATTCTGTTGGTCAAAAATAT
GTTTTTCCTTTTTTTGTGTGTGTTTTTTTTTTCCTTTAAAATGAACATATACTTCCAACATAGAAGTTG
TAACCTTTATATTTAACCAAGTTTCCAGTTGAAGCCAGTTTGGGGTGTGCATGTGTGTGCATGTGTCTATA
TGCGTGTGTGTGTATATACACACACACCAATTATATATATAGTATGCATGTGTGTATGTACATACAGAGAA
TTTTTGAGCTGGGGCCTTTTTAGCAGTAAAAAAAAAAAA

Figure 5

Figure 6

UNK2

GCTAGCATGGCCAACATGGTGAAACCCCGTCTCTACAAAAGAAAAAAAATACAAAAATTAGCTGGGTGTTGTGGTGTATGCCTGTA
ATCCCAACTATTTGGGTGGCTGAGGCACGAGAGTCGCTTGGACTTGGGGGGCGGAGGTTGCAGTGAGCTGAGATCGTGCCACTGC
ACTCCAGCCTGGGTGACAGACTGAGACAGTCTCAAAAAAAAAAAAAAAGAAAATAATGGATTTGCAGAGACTTGCTATTTAGATTT
CAGACATCTGTTAACTAAAACACATGTGTAGGCTTTTGTTACTTATTTCAGTAATCTGTAAATATCTTTATATTTGAGAAAATTT
GTGAGACATCTTTGTGTAAATTATAACTTGAAGAACCTCTCTTACAAGCAGGCATATTGGTAAGTAGCTGCGAGGATATAACTTA
TAACCAGATTGAAGTGTATAATTATAATATGTTATTATTCTGGGGTTCTATAAAAAATAAAATCTTTGAATCTAAAAAAAAAAAA

Figure 6

Figure 7

UNK3: 621 nt
GCTAGCAGACGACAAGAAATAACCAAGATCAGAGCTGAACTGAAGGAGATTGAGACACAAAAAAATTTAAA
AGATCAATGAATCCAGAAACTCATTCTTTGAAAAAACTCAGTAAAATAGACTGCTAGCTAGACTAATAAAA
AAGAAAAGAGAGAAGATTCAAATAAACACNATCAGAAGTAATAAGGGGGATAATACCACTGACCCCACAGA
ACTACAAACAACCATTAGAGGAGTCTATATNTATAAACTGGAAAATGTAGAAGAACTGGATACATTNCTGG
ACACGTACACCTCCCAAGACTGACCAGGAAGAATTGATCCCTGATAGACTAATTCATGGAATTCTGGAAAT
TGAGTCAGTAATAAATAGCTTACCAACCAGAAACAAGCCCAGGATCAGACAGATTCACAGCTAAATTCTAC
CAGATGTACAAAGAAGAGCTGATACTATTCCCACTGAAACTATTCCAAAAATTGAGGAGGAGGGACTCTTC
TCTAACATGCTATGAGGCCAGCATCATCCTAATACCAAAACCTGGTAGAGACACAACAAAAAAAAATAAAA
CTTCAGGCCAATATCCTTGATGAACATTGACGCAAAAATCCTAAAAAAAAAAAA

Figure 7

Figure 8

UNK4: 537 nt

GACCATTGCATTATAATGGAAGAGGGACCATATAAAGAGCCAGAATTACTGGGTGCTAATTCTAACCATGC
CTGGGCTGCCCTCATCTCTATGACTGTTCTTCCTGAGTTCTTTGCAGGTTAATTTCCTTTGTGTAGTCATA
AAATGATAAATTCGCCCTGAATAACAGTCTAGGCTCTACTCTAACCCCACACTATCTTCTGAGTAGGCTTA
CAAAGCCTAANNNTTACAAAGCNGAGNNGNATCNAGCTGTCAAAAGTGATTAGAAATTTTAAATGATCANT
CCAGCCTTTAATTTGGTATATGCACCATATTAAGTCATTTAAGTGAGTCAGTAAATGTGGCTTGTAATATA
AGAATGACAGTAATATCTATATGTGTATATTCTTTGATTGTCAGTGATGCATCAATTTACCAAAAACAGCA
GATAACAACTTAAAATATACTTTACTATTTTCAAATTGCAGTTTGATTAAGTGCAATTGCAATTGTACTTA
ATTGAAAAAAAATCAAGTTTTATATGAACAAAAAAAAAAAA

Figure 8

Figure 9

UNK5: 691 nt
GACCATTGCACAGAGCCCGGAGAAGGAAAGCAAGGATTATGAGATGAATGCGAACCATAAAGATGGTAAGA
AGGAAGACTGCGTGAAGGGTGACCCTGTCGAGAAGGAAGCCAGAGAAAGTTCTAAGAGAGCAGAATCTGGA
GACAAAGAAAAGGATACTTTGAAGAAAGGGCCCTCGTCTACTGGGGCCTCTGGTCAAGCAAAGAGCTCTTC
AAAGGAATCTAAAGACAGCAAGACATCATCTAAAGATGACAAAGGAAGTACAAGTNGTACTAGTGGTAGCA
GTGGAAGCTCAACTAAAAATATCTGGGTTAGTGAACTTTCATCTAATACCAAAGCTGCTGATTTGAAGAAC
TCTTTGGCAAATATGGAAAGGTTCTGAGTGCAAAAGTAGTTACAAATGCTCGAAGTCCTGGGGCAAAATGC
TATGGCATTGTAACTATGTCTTCAAGCACAGAGGTGTCCAGGTGTATTGCACATCTTCATCGCACTGAGCT
GCATGGACAGCTGATTTCTGTTGAAAAAGTAAAAGGTGATCCCTCTAAGAAAGAAACGAAGAAAGAAAATG
ATGAAAAGAGTAGTTCAAGAAGTCCTGGAGATAAAAAAAATACGAGTGATAGAAGTAGCAAGACACAAGCC
TCTGTCAAAAAAGAAGAGAAAAGATCGTCTGAGAAATCTCAAAAAAAAAAAA

Figure 9

Figure 10

UNK6: 392 nt
CGACTCCAAGCCCTGACTCTTTGCTGCGCCTGAGACAAAATAAACTTTCCATAAAAGACTGAGAATAGAAT
ACAAAGTAGTATACATAGCTATAACCAATAAACAAATTATGTCTTTAAAAATATCCCAAATGTGTGCAGAA
AAAAACATTAACAGTGACCGTCTTTGAGTAGTAGATATGACCAATATTATTCTCTTTGCTATAAATAGTAT
TCCAAATTTTAATAATACACTTTTTAAATATTTGTATACATACTTTTATATTTCACTATACTGTGTTGAAA
AGTATATATTGTAATAAGCTATTTTATACATGAAAGAAAAAAATTTTTGCATCATAAGTTGTATATATTAT
AATAAACTATTTTTAAGTTACCTTGAAAAAAAAAAAA

Figure 10

Figure 11

UNK7: 503 nt
GCTAGCATGGCAAGCGCCCATAAATGCCAGTAACTGTGGATGCTGCCAGAAGTCAGCTGTTTCCAGGGACA
CAGTGTAGCTGGGTTGCATTTTACAGTTTAATGTAACTCGGGTCGTCTTCTGTGGGAGTAAACTCATGTTT
TTGTGACTGTTTTATGGGTTTGTCCCTCATATTGGAGCTTAGTCTAAGCTGCGCCTCAGACTCCTGTGTCT
GTCATGCTGGGAGCCTTTGGAGAACGGTCCGTTTGTCCAACGTCCAGTTTGCTGAGCATTTTTAAATCCAA
CTCTGCACTTACACCTGGCCAGGCAGGAATGCTCCCAGAATGGGTCGGCAGTGTAGAAAGAGATCCTGAGA
AGTGGGTTTCTNTCTTTTGGTCAAAACTTACCTGTTTTGCATGAACATTTAAAAGTCTGTCTTGATCCCAA
TTTGGAACAATATGCCTCAAAACCATAAAGGTTGTATTTACCAGCCTGATGTTGATTTGACTAATGTTAAT
TTGCGAGAGATGAATATTAGTATCTTTTAAATAAAAAATGCCTGCCTATTTCACTATCAAAAAAAAAAAA

Figure 11

Figure 12

UNK8: 567 nt
GCTAGCATGGGTGATAGAGTGAGATCGTCTCCAAACTCTCCTTTCTGAAATTTTACTTAGCTAAATTTTTT
CCTAATTCCTCCTCAGTATTTCCACTTGATTCCCCCACAGAATGTAATTGTAATGTATTTATTAGTATCTT
TGAACATCTTTTATTATTTGCCTATCATACTTCTCTACAACAAAATATATGTAAGTTAATAAAAATATTTC
CTGTGTACATGATATTGTCTTAAATTTCTTCTATATTTAGTTATTACATTACATTTATTATTAGGACATGG
CAATTGAAGGAGCATAAATATACTTTGTTTTGCCAAACTAGTATGAAACATTTAAAAATGAAATTTTACTG
AATATATGCATTAGTGAGAAGGATGGTCCTTATTAATATAGTTGTAGGTGAATATTAAGCTAGAATGGTAG
TGTTCATTAATTCTCTCTTCCTATTTTCTATTTTTATATATGTGAATTCTAAAAAACCTTATTTACATAAT
GTTTTTAGTGCACATGGAAGTTTTTGATAACTTTTTAAATTGAATTTCTTCTGAATTATAAGTCAAAAAA

Figure 12

Figure 13

UNK9: 460 nt
GNTAGCAGACACATTTTCAAAGGGTCATATTCTTGGCTTGTTGGTAATCAGAATCGGGCAGGAGAAGTGGG
GTGGATGCAGACCAGCTGACCACACTGGCACCACCAGCAGTTTCAGTTTCGTCTTGATTGTAAAGAGGAAA
TATCTAATCTTAAAACTCATTAGGGGCCTGGCGCAGTGGCTCATACCTGTATTCCCAACACTTTGGGAGGC
CGAGGCAGGCAGATCACCCGAGGTCAGGATTTTGAGACCAGCCTGGCCAACATGGTGAAACCCCATCTCTA
CTAAAAATACAAAACTTAGCTAGGCGTGATGGCAGGCACCTCTAATCCCAGTTACTTGGGAGGCTGAGGCA
GGAGAATCACTTGAACCCGGAAGGCAGACGTTGCAGTGAGCCAAGATCGTGCCACTGCACTCCAGCCTGGG
CAACTAGAGCAAGACTCCATCTAAAAAAAAAAAA

Figure 13

Figure 14A

UNK10-5': 258 nt
GACCATTGCACAGAGCCCGGAGAAGGAAAGCAAGGATTATGAGATGAATGCGAACCATAAAGATGGTAAGA
AGGANGACTGCGTGAAGGGTGACCCTGTCGAGAAGGAAGCCAGAGAAAGTTCTAAGAAAGCAGAATCTGGA
GACAAAGAAAAGGATACTTTGAAGAAAGGGCCCTCGTCTACTGGGGCCTCTGGTCAAGCAAAGAGCTCTTC
AAAGGAATCTAAAGACAGCAAGACATCATCTAAAGATGACAAAGG

Figure 14B

UNK10-3': 259 nt
GGCATTGTAACTATGTCTTCAAGCACAGAGGTGTCCAGGTGTATTGCACATCTTCATCGCACTGAGCTGCA
TGGACAGCTGATTTCTGTTGAAAAAGTAAAAGGTGATCCCTCTAAGAAAGAAATGAAGAAAGAAAANGANG
AAAAGAGTAGTTCAAGAAGTTCTGGAGATAAAAAAATACGAATGATAGAAGTAGCAAGACACAAGCCTCTG
TCAAAAAAGAAGAGAAAAGATCGTNTGAGAAATCAAAAAAAAAAAA

Figure 14 A-B

Figure 15

UNK11: 696 nt
GGACCATTGCATTAAAATGTTTTGGATACCTGTTTGAATAACATTGCCTTAATGTTAATAAATCCATAATG
GTCACACAGGCAGGGGTGGTGTGTGAATCACCCTGGAAGGGATGTTCATTAATCAGTTACTTGGGGCTTTT
TTCTTTATTCATTCCCTCCTAGGGTTTGTACCTGTGAGGAAGCAGCCTACCTTCTTTGCAGCCATCTAGCA
TCTATATTCTAGAATCATTTTTCCCTATGATGGTCAAATCCAGATTATCTACACAGAAGAATAAAATAACC
TGAGTAATCCAAAGTGAGTCATAAGTTTTTAAAAGTCTGGGCCAGGCACAGTGTCTCATGCCTGTAATCCC
AGCATTTTAGGAGGCCCAGGAGGGAGGATCACTTGAGCTTAGGAGCTCGAGACCAGCCTGAGCAACATAGT
GAGACCCCATTTCTACCAAAAATAGTTTTAAAAATAGCCAGACATGGTGGTGCATCCCTGTGGTCCCAGGC
AGTTGGTGGCTGAGGTGGGAGGATCCTTTGAACCCAGGAGGTTGAGGTTGGAGTGAGCTATGATGGATCAC
ACCACTGCACTCCAGCCTGGGCAACCGAGTGAAACCCTTTCTCAAAAATATGCATTGTCCTTTGGAATATG
TTCTGTATTCGAACATGGATGTAGCTAATGTTTGATTTTAATTACAAAAAAAAAAAA

Figure 15

Figure 16

UNK12: 393 nt
GACCATTGCAAAATACTGTAGAAGAACTGTTTAGCTTGCTTCATTTCTTGGAACCGTCACAATTTCCCTCA
GAATCAGAGTTTCTCAAGGACTTTGGGGATCTCAAGACAGAGGAACAGGTTCAAAAGCTACAGGCCATTCT
TAAGCCAATGATGCTGAGAAGACTCAAAGAGGATGTTGAAAAAAACTTGGCACCCAAACAGGAAACAATTA
TTGAAGTAGAGCTGACTAATATCCAGAAGAAATACTATCGGGCTATTTTGGAGAAGAATTTCTCCTTCCTT
TCCAAAGGGGCAGGTCATACCAACATGCCTAATCTACTTAACACAATGATGGAGTTGCGCAAGTGCTGCAA
CCACCCATATCTCATCAATGGTGCTGAAAAAAAAAAAA

Figure 16

Figure 17

UNK13: 253 nt
GACCATTGCACAGTAAATCCATTGTAGGCTTTCTTTATGGGTGGCGGGGGAATCTCTAAAGGTCAGGAGTC
CAGATTGCTTCAAATAAACATCCAGAATCTCAGATGCTTTTTTGAAACAAGCCCAAGTTTATCTGAACCTC
TTTCTCTGGTTTGGAAATCAGGCTGAAAATGTCACAGAAACAGATTTTCTTGTGAGATCTCAGAATGTTGT
GGTTTAAGTAAAGTAATAAACAAAGTCGAAAAAAAAAAAA

Figure 17

Figure 18

UNK14: 307 nt
GGCCATTGCATAAAAGTAACTTTACAAGAATATTAGCACTAAATAATTCCTAACATCTTGGAGACAATTTT
CAAAATAATGCGTTTGTTTACTCATTCAAGATGTATTTACTGAGCTACCACTGTTATATGCCAAGTGATGT
TCTAGGTCCTAGACATGTAGCAAAAACCAAACTGAAAAAAAAAATTAACTCTTGTAGATTTTCAAAGCAACT
ATAGCAGCAAGAGGAAGAGACGAACACCGAAAAAAAAAAAAGCCCTATAGTGAGTCGTATTAAGCCGAATTC
TGCAGATATCCATCACACTGGCG

Figure 18

Figure 19A

UNK15-5': 481 nt
GACCATTGCAATGAATCCCCAATAATTGCAGAACTAAACTCATTTATAAAGCTAAAATAACCGGATATATA
CATAGCATGACATTTCTTTGTGCTTTGGCTTACTTGTTTAAAAAAAAAAAAAAACTAATCCAACCTGTTAGA
TTTNGCAGGTGAAGTCAGCAGCTTAAAAATGTCTTTCCCAGATTTCAATGATTTTTTTCCCCCTACCTCCC
AAAATCTGAGACTGTTAAAACATTTTTCTCCTATGAACACTGCTCAGACCTGCTTCGACATGCCATAGGAG
TGGCGTGCACATCTCTCTCTCTTCCAGCAGGAGGAGCCCGTGAGCACGCACAGCTGCCCTGTCTGCTCACC
CGAAGGCACCGGGCTCACCTGGACCTCCCAGGAAAGGGAGAAAGAGCCTCCAGAAACTGCTCTGTGTTTAG
AAAGGAATATCTTTAAGAATCCAAGTTTTTCATTTCCACAAATTTCCTATATCCA

Figure 19B

UNK15-3': 450 nt
GATTTGTTTGGACAATGTAGTTGGGAAGAACTAAGATTCTAATCTGTGAAGAACCTTATAGGGCCTTCTAA
AACATAAGAGTTTCCTTTGTTGCTTCAAATATTTGAACATTATGTTAAAGATCAAGTATTAATTTTAGTTG
TACTCTAGAAAGCTAAAGTGCCACATTCGGGGCTATTTTTATGATTCAGCAATCTTTTCTAAATTGTGTAG
CATGTGTATGAGACTATTTATACCCAAGGATATGAAGGAATATAAGTGACTACAAGGCTCTAATAAGCCAC
GGTGGCAGGAGGTTCAAGCGGTTCTGTTCACTAAATTTTTCTCCTGTAAGCTTTGAATGGAAACTTCTGTA
TCACATGATGTGTTTCACTTATGCTGTTGTGTATATACCTAATATTTCTATTTTTGATTTTATTTTAATAC
ACCTCGTCCAATAAAAAAAAAAAA

Figure 19 A-B

Figure 20A

UNK16-5': 420
GCTAGCAGACCCACTTAAGGATGAATTAAACCTTGCTGATTCTGAAGTGGATAACCAAAAACGAGGGAAAC
GACNTTATGAAGAAAAACAAAAAGAACACTTGGATACCTTAAATAAAAAGAAACGAGAACTGGATATGAAA
GAGAAAGAACTAGAGGAGAAAATGTCACAAGCAAGACAAATCTGCCCAGAGCGTATAGAAGTAGAAAAATC
TGCATCAATTCTGGACAAAGAAATTAATCGATTAAGGCAGAAGATACAGGCAGAACATGCTAGTCATGGAG
ATCGAGAGGAAATAATGAGGCAGTACCAAGAAGCAAGAGAGACCTATCTTGATCTGGATAGTAAAGTGAGG
ACTTTAAAAAAGTTTATTAAATTACTGGGAGAAATCATGGAGCCCAGATTCCAGACATATCAACC

Figure 20B

UNK16-3': 507 nt
GACCACAAGATGAAATTCTAAGTATATCAGTTCAGCCTGGAGAAGGAAATAAAGCTGCTTTCAATGACATG
AGAGCCTTGTCTGGAGGTGAACGTTCTTTCTCCACAGTGTGTTTTATTCTTTCCCTGTGGTCCATCGCAGA
ATCTCCTTTCAGATGCCTGGATGAATTTGATGTCTACATGGATATGGTTAATAGGAGAATTGCCATGGACT
TGATACTGAAGATGGCAGATTCCCAGCGTTTTAGACAGTTTATCTTGCTCACACCTCAAAGCATGAGTTCA
CTTCCATCCAGTAAACTGATAAGAATTCTCCGAATGTCTGATCCTGAAAGAGGACAAACTACATTGCCTTT
CAGACCTGTGACTCAAGAAGAAGATGATGACCAAAGGTGATTTGTAACTTAACATGCCTTGTCCTGATGTT
GAAGGATTTGTGAAGGGAAAAAAAATTCTGAACTCTTTGATATAATAAAATGAGACTGGAGGCATTCTCAA
AAAAAAAAAA

Figure 20 A-B

Figure 21A

UNK17-5': 513 nt
GCTAGCAGACTATCATTAACCAAATAAATTATGGGATTTTGTCTTAATTATATACATATACATATACACAC
ACATACACATACACATACATGTGTATATATTCCCTAAAACTTAATAAAGCTCAAATAATAAAATCAGATTT
CTTAAGTATTCCAATTCCCTTTAAAATGTAAATCAGATTTTATAATTCTTTTGTTCAAAACTGTCCATTGG
CTCCCATTTCACTTAAATCAAAAGCTAGTTTTTACAATAAGCTAAGGTAGCAAACATTATTATCTATTTAC
TTATGAGTTACTTATGTAACTCAGCATCCAATAACACTGTAGGTGCTCAATAAAATAGTTGCTGAATGGAT
AACTTTCACTATTTGGATGAGATCCAACAGAAAAGAATACTCTTAGCTTGACAAACAATGGTAAACAGAAG
TTAACATTAGAACACTAGATCCTTGCTCACTAAAATCAGACATAATTATATGTTTGTGTGTGTGTGTAAAT
ATAAACGTATATATGT

Figure 21B

UNK17-3': 489 nt
TACATATTTGAATTAAATGAAATATATCAGAATTTGTGGTAACAACGGATTAAAGCTTAGTTCAGAAAAGA
AGAAAGTTTTCAAATCAGCGATATAATAATTTCCAAACTTAAGAAACTAGAAGAGCAAATTGAACCAAAGC
AGGCAGAATGGAAGAAAGAATAAGATAAGAAAATCAATGAAATTAAAAGCAACAGAAACTAAGGCCAGGTG
CAGTGGCTCATGCCTGTAATCCCAACACTTCGGGAGGCCGAGGTGGGCAGGTCACGTGAGGTCAGGAGTTT
GAGACCAGCCTAACCATCATGGCAAAACCATCTCTACTAAAAATACAAAAATAAGCTGGGCATGGTGGCAG
GCACCAGTAATCCCAGCTACTCGGGAGACTGAGGCAGAAGAATCACTCTGGGAGGCAGAGGCTGTAGTGAG
CTGAGATTGCCACTGCACTCTAGCCTGGGCTACAGAGTGAGACTCCATCTCAAAAAAAAAAAA

Figure 21 A-B

Figure 22

UNK18: 505 nt
GGTTTTTTGAAAGGATCAACAAAATTGATAGATCTCTAGCAAGACTAATAAGAAAAGAGAGAAGAATCAAG
TGGATGCAATAAAAAATGATAAAGGGGATATCACCACTGATTCCATAGAAGTACAAACTACCATCAGAGAA
TACTACAAACACCTCTACACAAATAAACTAGAAAATCTAGAAGAAATGGATAAATTCCTGGACACATACAC
CCACCCAAGACTAAACCAGGAAGAAGTTGAATCTCTGAATAGACCAGTAACAGGCTCTGAAATGGAGGCAA
TAATTAATAGCTTACCAACCAAAAAAAGTCCAGGACCAGATGGAATCACAGCTGAATTCTGTCAGAGGTAC
AAAAAGGAGCTGGTACCATTCCTTCTGAAACTATTCCAATCAATAGGAAAAGAGGGAATCCTCCCTAACTC
ATTTTATGAGGCCAGCACCATCCTGATACCAAAGCCTGGCAGAGACGCGACAAAAAGAATTTTACACCAAA
AAAAAAAA

Figure 22

Figure 23

UNK19: 506 nt
GCTAGCAGACGGCGAGAAATAACTAAAATCAGAGCACAACTGAAGGAAATAGAGACACAAAAAACCCTTCA
AAAAATTAAGGAATCCAGGAGCTGGTTTTTTGAAAGGATCAACAAAATTGATAGACCACTAGCAAGACTAA
TAAAGAAGAAAAGAGAGAAGANTCAAATAGACGCAATAAAAAATGATAAAGGGGAAATCACCACCAATCCC
ACAGAAATACAAACTACCATCAGAGAATACTACAAACACCTCTATGCAAATAAACTAGAAAATCTAGAAGA
AATGGATAAATTCCTCGACACATACACCCTCCCAAGACTAAACCAGGAAGAAGTTGAATCTCTGAATAGTC
CAATAACAGGCTCTGAAATTGTGGCAATAATCAATAGCTTACCAACCAAAAAGAGTCCAGGACCAGATGGA
TTCACAGCCAAATTCTACCAGANGTTTAAGGAAGAACTGGTACCATTCCTTCTGAAACTACTCTAATCNAT
AGAAAAAGA

Figure 23

Figure 24

UNK20: 488 nt
GCTAGCAGACCACAAAGGACGTTGATCCCTGAGGGAGGTGAATCCTATGAAGGCTCTGGCTTAAGCCCGTG
AAGGTTTCTTAGCAGTGGCACAGGAAGGGCAACTAACTCAAGGGAGGCAAACTCCATGAATTGAGGAAACA
GAGCTAAAGATATGGGATAATAAAGTAGCTAGAGCTTACAGGACAGAGTTCAGGAGAGAGCAGATGCACAG
ACAACAATCTCTTGAAATCTGCAGAGAGTCTCAGAGATCTGGATATGTGCATGAGGAAGCTACCCAAGGCT
GAGGAAAGAAGCATCGGAAATAATTATACGGGGAACAGTACCTGGCACCTTCCTAGGGCTGGAAATAGGCC
TTTTCTCACCAGTCAGAATGGAAAATCTCTTAATTCATATGCAATTAGGTAGAACCTCAGTAGTGAGAAAT
GAGGTAGACTATGCACTGCTCTGGTCTCTCCTAGCTAACATTTTAATCCCAAAAAAAAAAAA

Figure 24

Figure 25A

UNK21-5': 267 nt
ATGGTAGTCTCATCACACACTACAATTACCTTCCCTTACATTACTAATTTGAAGCATAATTAACACAAGCC
TCACATACTTGGTAAAGTTTGCTATGTTATAGTTAAAGTCTGTCTTCACAGATCACTACGTTTGTGACTCA
TTGCGAGTTCAATAATCAAAGTTCATGAACTCGAGGTGATTGATACACAGTGTCCTCATCAGTGAACCTGG
TGTTAATGTAGTATTTGTCCAGAAAGTTATTGTGAGGACTGTATAAACCCTTGC

Figure 25B

UNK21-3': 309 nt
GTAAAATGGGTGATAACAGTAGCAAATTCCAGGTATTGCTGTGAGATAATAGGGTACTTAGAACAGGGCTT
AACACTTAGTATTGCATAGTCATTATTTGCTGTTATTAAAGAATAATGTTTTGGAAAGGGCCTGGCACATA
AAAAAGCTATTAATATTAAATACTATTATTAGTATCAAGAATAAAAGATTAGATATCACTACTGGTTCTAC
ATTCAGTAAAGAATAACATGATAATTTACAAATAATGTTATGACAATAAGCCTGACAACTTAAATAAAAAT
GACAAATCCCTCGAAAAAAAAAAAA

Figure 25 A-B

Figure 26A

UNK22-5': 217 nt
ATGGTAGTCTAAGTAAAAAAAAAAAAAGCCCTATAGTGAGTCGTATTACAAGCCGAATTCCAGCACACTGGC
GGCCGTTACTAGTGGATCCGAGCTCGGTACCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGA
AATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAACCCTGGGGTCCCTA
ATGA

Figure 26B

UNK22-3': 349 nt
GTAACCCACCACACCCGCGGCGGTTAATGGGCCGCTACAGGGCGCGTCCATTCGCCATTCAGGCTGGCGCA
ACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCA
AGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTGTA
ATACGACTCACTATAGGGCGAATTGGGCCCTCTAGATGCATGCTCGAGCGGCCGCCAGTGTGATGGATATC
TGCAGAATTCGGCTTAGCGGATAACAATTTCACACAGGAATGGTAGTCTAAGTAAAAAAAAAAAA

Figure 26 A-B

Figure 27

UNK23: 433 nt
ATGTAGTCTACATTTGACATACACTGGTGACATTCAAAGGTATAGTTCTGGTAAAATAAAATTGAACATAT
GGTGGCACCAGCACTGAGAGCTTGGTTCTTTTCCTGATCAGCAGTTTGGCTCTTCATCAGTTAACTGCCTG
GGCCTCAGTTTCTCAGCTGTTAAATTGAAGGAGGTGGATGAGTTATAACGTTCCTTTCTAGTTCTTACACA
GAATGAGTTTCTTGAGTTCCAATATGCTGGAGAAGAAAAATAGAAGAGTTTGGCCACTAATTTATAACAGA
AGTAGTATATACCAGGACACGTGATAAATTATAGACATTTTCTGTTAGGGAGACTTGTCTGAAGACTAGTT
TTATTACTTTCATTTCTTCCTCAAAGATCCTTTCATAAAAAACAAACAAACAAAAAACAAAAAACGAAAAA
AAAAAAA

Figure 27

Figure 28

UNK24: 222 nt
ATGGTAGTCTAATCATCAGAGAAATTACAGCTGTAGTGAAATTGTGATGAAGATAATGTTGGATTGACTAC
CTACCAGCATACCTGAGACATAGTCGATGCTCAATGATATTAGGTCCTTTCTGTAATGAAAAAATCTCGTA
TATTCCAATCCCCTTTTTCACCAATTTATGAACATGTGNGTATGTGTTTATAAACACACATGTGCTTGTGT
GATTTGGGG

Figure 28

Figure 29A

UNK25-5': 337 nt
ATGGTAGTCTAGGAGAAGAGAGGGCTCACANCCAGACACACCTGGGTGGGGCTNGGGTCAAGTGTCTTCAT
CTCTCTGAGTCTATCTCCCCAACTTTTAAAAACAGACAGTGTATGTACNACATAGGAGGGGCTCTCATAAC
TGCCATCCCTTCCACTTTCCTAACTTTGCCCCCATACACCCTCACCCCCATCAAGCCCTTGCCCAGGACAG
ATTTGGACAGCTCTCCTCTACTCAGATACGAAAACAAAAAAAAGCCCTATAAGCCGAATTCTGCAGATATC
CATCACACTGGNCGGCCGCTCGAGCANCNCATCTAGAGGGCCCAATTCGCCCT

Figure 29B

UNK25-3': 89 nt
TTTGCCCCCATACACCCTCACCCCCATCAAGCCCTTGCCCAGGACAGATTTGGACAGCTCTCCTCTACTCA
GATANAAAAACCAAAAAA

Figure 29 A-B

Figure 30A

UNK26-5': 298 nt
CAGCCNCAGNACACACCTGGGTGGGGCTNGGGNCAAGNGTCTNCATCTCTCNGAGNCTATCTCCCCAACNN
NNAAAAACAGACAGCGNATGNACTACATAGGAGGGGCTCTCATAACTGCCATCCCNTCCACTNNNCTAACT
NNGCCCCCATACACCCTCACCCCCATCAAGCCCTTGCCCAGGACAGACNNGGACAGCTCTCCCCTACTCAG
ACACGAAAAAAAAAAAAAGCCCTATAGNGAGNCGCANAACAAGCCGAACNCNGCAGANATCCATCACACNGG
CGGCCGCCCGAGCA

Figure 30B

UNK26-3': 85 nt
CCCCCATACACCCTCACCCCCATCAAGCCCTTGCCCAGGACAGATTTGGACAGCTCTCCTCTACTCAGATN
CGAAAAAAAAAAAA

Figure 30 A-B

Figure 31

UNK27: 684 nt
ATGGTAGTCTTAAGTCAACTTTGACAGGAAATAAAGTGTTTAATTGTATTTCCTATTCTCCACTTTGTAAA
CGTTTAGCATCTGGAAGCACAGATAGGCATATCAGACTGTGGGATCCCCGAACTAAAGATGGTTCTTTGGT
GTCGCTGTCCCTAACGTCACATACTGGTTGGGTGACATCAGTAAAATGGTCTCCTACCCATGAACAGCAGC
TGATTTCAGGATCTTTAGATAACATTGTTAAGCTGTGGGATACAAGAAGTTGTAAGGCTCCTCTCTATGAT
CTGGCTGCTCATGAAGACAAAGTTCTGAGTGTAGACTGGACAGACACAGGGCTACTTCTGAGTGGAGGAGC
AGACAATAAATTGTATTCCTACAGATATTCACCTACCGCTTCCCATGTTGGGGCATGAAAGTGAACAATAA
TTTGACTATAGAGATTATTTCTGTAAATGAAATTGGTAGAGAACCATGAAATTACATAGATGCAGATGCAG
AAAGCAGCCTTTTGAAGTTTATATAATGTTTTCACCCTTCATAACAGCTAACGTATCACTTTTTCTTATTT
TGTATTTATAATAAGATAGGTTGTGTTTATAAAATACAAACTGTGGCATACATTCTCTATACAAACTTGAA
ATTAAACTGAGTTTTACATTTCTCTTTAAAGGTAAAAAAAAAAAA

Figure 31

Figure 32

UNK28: 694 nt
ATGGTAGTCTGTCCAGTGGATAAGGTGTTTCTCTCACTTTTTATGTAACAACTGAGTAATGACAACAAAGT
TTACCTACCACTCCTTAGGATATAAGGCCCAGTAAGGCAGAGTTTTTGTTTTTCTTTTTTCCTACTTTATT
CACTGCTATGTCCCAGCCCCTAGAACAACTAGTTACAACTAGGCAGTTGTAACTGCCTAGTACATAATAGG
GACTCAAAAATATTTGTAAATGAATGAATAAATCCACTTTCCCAGAATTACCAAGGCACATATTTCTGTTG
TCAGAAGTAGAGACTCTTAAACTTTGTTGTACATCAGAACCACAGATGCAGCATCTTAATGTACATGTCCC
TTCCCCAGCCCTAGTTTACTGTATGTGTATTTGGAAAGGAATCCACAGATGATTCTGACATGTGAAAGGCT
AAGAAGCAGGGAGCTTGCCAGGAAGGTTGAAATTAAAATCTGAAAGTTGTGGGGAGTCTTAGAATATTAAG
TGTTACTTTTTGTTGGAAATGGCTTCTTTTGTCTTTATTAAAGTTAGGAATGTGTTTTCTGAAAAGCTTAC
TTTTTGATATTAATTTCCATTTTTAAAGAAATAACTTGAGATTACAGGCGTGAACCACCGCGCCCGGCCGA
CTTCAGGAGATCTTTAGGCATCATTGGTTTGTGTTCTTCAGGTAAAAAAAAAAAA

Figure 32

Figure 33

```
UNK19       GCTAGCAGAC GGCGAGAAAT AACTAAAATC AGAGCACAAC TGAAGGAAAT

UNK19       AGAGACACAA AAAACCCTTC AAAAAATTAA GGAATCCAGG AGCTGGTTTT
|||                                                       ||||||
UNK18                                                     GGTTTT

UNK19       TTGAAAGGAT CAACAAAATT GATAGACCAC TAGCAAGACT AATAAAGAAG
|||         |||||||||| |||||||||| |||||||| | |||||||||| ||||| |
UNK18       TTGAAAGGAT CAACAAAATT GATAGATCTC TAGCAAGACT AATAA-G---

UNK19       AAAAGAGAGA AGANTCAAAT AGACGCAATA AAAAATGATA AAGGGGAAAT
|||         |||||||||| ||| |||||| || |||||| |||||||||| ||||||| ||
UNK18       AAAAGAGAGA AGAATCAAGT GGATGCAATA AAAAATGATA AAGGGGATAT

UNK19       CACCACCAAT CCCACAGAAA TACAAACTAC CATCAGAGAA TACTACAAAC
|||         |||||| ||  ||| ||||  |||||||||| |||||||||| ||||||||||
UNK18       CACCACTGAT TCCATAGAAG TACAAACTAC CATCAGAGAA TACTACAAAC

UNK19       ACCTCTATGC AAATAAACTA GAAAATCTAG AAGAAATGGA TAAATTCCTC
|||         ||||||| | |||||||||| |||||||||| |||||||||| |||||||||
UNK18       ACCTCTACAC AAATAAACTA GAAAATCTAG AAGAAATGGA TAAATTCCT-

UNK19       GGACACATAC ACCCTCCCAA GACTAAACCA GGAAGAAGTT GAATCTCTGA
|||         |||||||||| |||||||||| |||||||||| |||||||||| ||||||||||
UNK18       GGACACATAC ACCCACCCAA GACTAAACCA GGAAGAAGTT GAATCTCTGA

UNK19       ATAGTCCAAT AACAGGCTCT GAAATTGTGG CAATAATCAA TAGCTTACCA
|||         |||| ||| | |||||||||| ||||| | || ||||||| || ||||||||||
UNK18       ATAGACCAGT AACAGGCTCT GAAATGGAGG CAATAATTAA TAGCTTACCA

UNK19       ACCAAAAAGA GTCCAGGAC CAGATGGATT CACAGCCAAAT TCTACCAGAN
|||         |||||||| | ||||||||| |||||||| | |||||| ||| ||| ||||
UNK18       ACCAAAAAAA GTCCAGGAC CAGATGGAAT CACAGCTGAAT TCTGTCAGAG

UNK19       GTTTAAGGAA GAACTGGTAC CATTCCTTC TGAAACTACTC TAATCNATAG
|||         ||  ||  |  || ||||||| |||||||| |||||||| ||  |||| ||||
UNK18       GTACAAAAAG GAGCTGGTAC CATTCCTTC TGAAACTATTC CAATCAATAG

UNK19       AAAAAGA
|||          ||||||
UNK18       GAAAAGAGGG AATCCTCCCT AACTCATTTT ATGAGGCCAG CACCATCCTG

UNK18       ATACCAAAGC CTGGCAGAGA CGCGACAAAA AGAATTTTAC ACCAAAAAAA

UNK18       AAAA
```

Figure 33

Figure 34

```
UNK5        GACCATTGCA CAGAGCCCGG AGAAGGAAAG CAAGGATTAT GAGATGAATG
|||         |||||||||| |||||||||| |||||||||| |||||||||| ||||||||||
UNK10-5'    GACCATTGCA CAGAGCCCGG AGAAGGAAAG CAAGGATTAT GAGATGAATG

UNK5        CGAACCATAA AGATGGTAAG AAGGAAGACT GCGTGAAGGG TGACCCTGTC
|||         |||||||||| |||||||||| ||||| |||| |||||||||| ||||||||||
UNK10-5'    CGAACCATAA AGATGGTAAG AAGGANGACT GCGTGAAGGG TGACCCTGTC

UNK5        GAGAAGGAAG CCAGAGAAAG TTCTAAGAGA GCAGAATCTG GAGACAAAGA
|||         |||||||||| |||||||||| |||||||||| |||||||||| ||||||||||
UNK10-5'    GAGAAGGAAG CCAGAGAAAG TTCTAAGAAA GCAGAATCTG GAGACAAAGA

UNK5        AAAGGATACT TTGAAGAAAG GGCCCTCGTC TACTGGGGCC TCTGGTCAAG
|||         |||||||||| |||||||||| |||||||||| |||||||||| ||||||||||
UNK10-5'    AAAGGATACT TTGAAGAAAG GGCCCTCGTC TACTGGGGCC TCTGGTCAAG

UNK5        CAAAGAGCTC TTCAAAGGAA TCTAAAGACA GCAAGACATC ATCTAAAGAT
|||         |||||||||| |||||||||| |||||||||| |||||||||| ||||||||||
UNK10-5'    CAAAGAGCTC TTCAAAGGAA TCTAAAGACA GCAAGACATC ATCTAAAGAT

UNK5        GACAAAGGAA GTACAAGTNG TACTAGTGGT AGCAGTGGAA GCTCAACTAA
|||         ||||||||
UNK10-5'    GACAAAGG

UNK5        AAATATCTGG GTTAGTGAAC TTTCATCTAA TACCAAAGCT GCTGATTTGA

UNK5        AGAACTCTTT GGCAAATATG GAAAGGTTCT GAGTGCAAAA GTAGTTACAA

UNK5        ATGCTCGAAG TCCTGGGGCA AAATGCTATG GCATTGTAAC TATGTCTTCA
|||                                       | |||||||||| ||||||||||
UNK10'3'                                  G GCATTGTAAC TATGTCTTCA

UNK5        AGCACAGAGG TGTCCAGGTG TATTGCACAT CTTCATCGCA CTGAGCTGCA
|||         |||||||||| |||||||||| |||||||||| |||||||||| ||||||||||
UNK10-3'    AGCACAGAGG TGTCCAGGTG TATTGCACAT CTTCATCGCA CTGAGCTGCA

UNK5        TGGACAGCTG ATTTCTGTTG AAAAAGTAAA AGGTGATCCC TCTAAGAAAG
|||         |||||||||| |||||||||| |||||||||| |||||||||| ||||||||||
UNK10-3'    TGGACAGCTG ATTTCTGTTG AAAAAGTAAA AGGTGATCCC TCTAAGAAAG

UNK5        AAACGAAGAA AGAAAATGAT GAAAAGAGTA GTTCAAGAAG TCCTGGAGAT
|||         |||||||||| |||||||||| |||||||||| |||||||||| ||||||||||
UNK10-3'    AAATGAAGAA AGAAAANGAN GAAAAGAGTA GTTCAAGAAG TTCTGGAGAT

UNK5        AAAAAAAATA CGAGTGATAG AAGTAGCAAG ACACAAGCCT CTGTCAAAAA
|||         ||||||| || |||||||||| |||||||||| |||||||||| ||||||||||
UNK10-3'    AAAAAAA TA CGAATGATAG AAGTAGCAAG ACACAAGCCT CTGTCAAAAA

UNK5        AGAAGAGAAA AGATCGTCTG AGAAATCTCA AAAAAAAAAA A
|||         |||||||||| |||||||||| |||||||||| |||||||||
UNK10-3'    AGAAGAGAAA AGATCGTNTG AGAAATCAAA AAAAAAAAA
```

Figure 34

Figure 35

```
Factor B:   356   NFWVSGLSSTTRATDLKNLFSKYGKVVGAKVVTNARSPGARCYGFVTMSTAEEATKCINH
      415
                  N WVSGLSS T+A DLKNLF KYGKV+ AKVVTNARSPGA+CYG VTMS++ E ++CI H
AK000867:   385   NIWVSGLSSNTKAADLKNLFGKYGKVLSAKVVTNARSPGAKCYGIVTMSSSTEVSRCIAH
      444
                  N+WVSGLSS T+ ADLKNLF KYGKV+ AKVVTNARSPGA+CYG VTMS+S E ++CI+H
KIIA0138:   408   NLWVSGLSSTTRATDLKNLFSKYGKVVGAKVVTNARSPGARCYGFVTMSTSDEATKCISH
      467

consensus:        NlWVSGLSStTrAtDLKNLFsKYGKVvgAKVVTNARSPGArCYGfVTMStseEatkCIaH


Factor B:   416   LHKTELHGKMISVEKAKNEPVGKKTSDKRDSDGKKEKSSNSDRSTNLKRDDK
      467
                  LH+TELHG++ISVEK K +P    K    K+++D K    S+  D+      R  K
AK000867:   168   LHRTELHGQLISVEKVKGDPSKKEMKKENDEKSSSRSSGDKKNTSDRSSKTQASVK
      001
                  LHRTELHG++ISVEK K +P+ K+++KE + KSS      D+++
KIIA0138:   468   LHRTELHGRMISVEKAKNEPAGKKLSDRKECEVKKEKLSSVDRHHS
      513

consensus:        LHrTELHGkmISVEKaKnEPagKKmSDkndeKSSkekssdvdr


consensus:

NlWVSGLSStTrAtDLKNLFsKYGKVvgAKVVTNARSPGArCYGfVTMStseEatkCIaHLHrTELHGkmISVEKaKnEPagKKm
SDkndeKSSkekssdvdr
```

Figure 35

Figure 36

GACCATTGCATCATTGGCCGCACACTGGTGGTCCATGAAAAAGCAGATGACTTGGGCAAAGGTGGAAATGG
AGAAAGTACAAAGACAGGAAACGCTGGAAGTCGTTTGGCTTGTGGTGTAATTGGGATCGCCCAATAAACAT
TCCCTTGGATGTAGTCTGAGGCCCCTTAACTCATCTGTTATCCTGCTAGCTGTAGAAATGTATCCTGATAA
ACATTAAACACTGTAATCTTAAAAGTGTAATTGTGTGACTTTTTCAGAGTTGCTTTAAAGTACCTGTAGTG
AGAAACTGATTTATGATCACTTGGAAGATTTGTATAGTTTTATAAAACTCAGTTAAAATGTCTGTTTCAAT
GACCTGTATTTTGCCAGACTTAAATCACAGATGGGTATTAAACTTGTCAGAATTTCTTTGTCATTCAAGCC
TGTGAATAAAAACCCTGTATGGCACTTATTATGAGGCTATTAAAAGAATCCAAATTCAAAATAAAAAAAAA
AA

Figure 36

CLONE	Comments
UNK1	Genbank nt AC004805 Homo sapiens Xp22 bins 16-17 BAC GSHB-531I17 (Genome Systems Human BAC Library) (E -6) Genbank nr Human EST Unigene
UNK3	Genbank nt HSJ757N13 Human DNA sequence from clone RP4-757N13 on chromosome 1p13.1-13.3 (E-43) several others Genbank nr 2072972 (U93572) putative p150 [Homo sapiens] (E-42)) several others Human EST AA904136 od88a04.s1 NCI_CGAP_Br5 Homo sapiens cDNA Unigene Hs#S364404 za24f06.s1 Homo sapiens cDNA (E-27)
UNK4	Genbank nt No significant matches Genbank nr

FIGURE 37A

Human EST

Unigene

UNK5

Genbank nt

Genbank nr
1213639 (L43631) scaffold attachment factor B [Homo sapiens] (E -27)

Human EST
AA157075 zo51f03.s1 Stratagene endothelial cell 937223 Homo sapiens (- perfect match)

Unigene
Hs#S989538 om88c01.s1 Homo sapiens cDNA, 3' end (- perfect match)

UNK6

Genbank nt
No significant matches

Genbank nr

Human EST

Unigene

UNK7

Genbank nt

FIGURE 37B

	No significant matches
	Genbank nr
	Human EST
	Unigene
UNK8	**Genbank nt** **No significant matches**
	Genbank nr
	Human EST
	Unigene
UNK9	**Genbank nt**
	Genbank nr **P39194| ALU Sequence** **3002527 (AF010144) neuronal thread protein AD7c-NTP (E -17)**
	Human EST **AI433131.1|AI433131 th41g10.x1 NCI_CGAP_Lym12 Homo sapiens (E -83)**
	Unigene **Hs#S813305 nj25f01.s1 Homo sapiens cDNA (E -73)** **Human NAD(P)H:quinone oxireductase gene (E -68)**

FIGURE 37C

UNK10_5'	Genbank nt
	Genbank nr
	Human EST AA133526 zo14h11.r1 Stratagene colon (#937204) Homo sapiens cDNA clone 586917 5' similar to TR:G1213639 G1213639 SCAFFOLD ATTACHMENT FACTOR (E -108)
	Unigene
UNK10_3'	Genbank nt
	Genbank nr NP_002958.1|PSAFB| scaffold attachment factor B >gi|2828537 (U72355) Hsp27 ERE-TATA-binding protein [Homo sapiens] (E -8) Human EST AA594603|AA594603 nl99a05.s1 NCI_CGAP_Co10 Homo sapiens cDNA clone IMAGE:1058768 similar to TR:G1213639 G1213639 SCAFFOLD ATTACHMENT FACTOR (eE -112)
	Unigene Hs#S989538 om88c01.s1 Homo sapiens cDNA (E -113)
UNK11	Genbank nt

FIGURE 37D

AL023802.1|HS983L19 Human DNA sequence from clone RP5-983L19 on chromosome 22q13.31-33Contains a 60S Ribosomal Protein L5 LIKE pseudogene, ESTs, an STS, GSSs and a CpG Island, complete sequence (E -38)

Genbank nr
ALU sequence
3002527 (AF010144) neuronal thread protein AD7c-NTP (E -8)

Human EST
AI688963 tx91c11.x1 NCI_CGAP_Ut4 Homo sapiens cDNA clone IMAGE:2276948 3'similar to contains Alu repetitive element;contains
element LTR5 repetitive element (E -32)

Unigene
Hs#S273654 yr36e12.r1 Homo sapiens cDNA (E -23)
Hs#S377539 Homo sapiens semaphorin F homolog mRNA (E -19)
Hs#S1090638 Homo sapiens protease-activated receptor (E -19)

UNK12

Genbank nt

Genbank nr
(AL031667) dJ620E11.1a (novel Helicase C-terminal domain and SNF2
N-terminal domains containing protein, similar to KIAA0308) [Homo sapiens] (E -62)

Human EST

FIGURE 37E

	AI659009 tn23e08.x1 NCI_CGAP_Pr28 Homo sapiens cDNA clone IMAGE:2251910 3' similar to gb:M64716 40S RIBOSOMAL PROTEIN S25 (HUMAN) (E -47)
	Unigene
UNK13	**Genbank nt** **No significant matches**
	Genbank nr
	Human EST
	Unigene
UNK14	**Genbank nt**
	Genbank nr
	Human EST **R96185|R96185 yt84g10.s1 Homo sapiens cDNA clone 231042 (E -91)**
	Unigene **Hs#S572202 Homo sapiens alpha1A-voltage-dependent calcium channel mRNA (E -3) not very significant**
UNK15_3'	**Genbank nt**

FIGURE 37F

	AF000302|RNAF000302 Rattus norvegicus Lyn B tyrosine kinase (E -15) Genbank nr Human EST AW024939 wu70a06.x1 NCI_CGAP_Kid3 Homo sapiens cDNA clone (- perfect match) Unigene Hs#S1237857 qz26h02.x1 Homo sapiens cDNA (- perfect match)
UNK15_5'	Genbank nt NM_002350.1|LYN| Homo sapiens v-yes-1 Yamaguchi sarcoma viral related oncogene homolog (LYN) mRNA >gi|187268|gb|M16038|HUMLYN Human lyn mRNA encoding a tyrosine kinase (E -54) Genbank nr Human EST AI701165.1|AI701165 we10g08.x1 NCI_CGAP_Lu24 Homo sapiens (- perfect match) Unigene Hs#S1237857 qz26h02.x1 Homo sapiens cDNA (E -71)
UNK16_3'	Genbank nt

FIGURE 37G

	Genbank nr P53692|RA18_SCHPO DNA REPAIR PROTEIN RAD18 >gi|1150622|emb|CAA56900| (X80929) rad18 [Schizosaccharomyces pombe] >gi|3859084|emb|CAA21961| (AL033406) dna repair protein rad18 (E -20)
	Human EST AI911463 wd25d06.x1 Soares_NFL_T_GBC_S1 Homo sapiens cDNA clone IMAGE:2329163 3' similar to SW:RA18_SCHPO P53692 DNA REPAIR PROTEIN RAD18. ; (- perfect match)
	Unigene Hs#S440252 zh81c02.s1 Homo sapiens cDNA (E-6)
UNK16_5'	Genbank nt
	Genbank nr NP_013487.1|RHC18| involved in recombination repair Saccharomyces cerevisiae (E -3)
	Human EST AA811277 ob68e06.s1 NCI_CGAP_GCB1 Homo sapiens E -38)
	Unigene
UNK17_3'	Genbank nt AL132857.2|CNS01DTS Human chromosome 14 DNA sequence (E -68)
	Genbank nr ALU sequences

FIGURE 37H

	Human EST AA328234 EST31692 Embryo, 12 week I Homo sapiens cDNA 5' end similar to ESTcontaining Alu repeat
	Unigene Hs#S1292073 ta13b12.x1 Homo sapiens cDNA (E -42)
UNK17_5'	Genbank nt No significant matches
	Genbank nr
	Human EST
	Unigene
UNK18	Genbank nt AC006061 Homo sapiens Xp22-182-183 BAC GSHB-19O24 (-perfect match)
	Genbank nr 072964 (U93569) putative p150 [Homo sapiens] (E -74)
	Human EST AA194957 zr35a10.s1 Soares NhHMPu S1 Homo sapiens cDNA clone 665370 3'similar to gb:M19503 LINE-1 REVERSE TRANSCRIPTASE HOMOLOG (HUMAN);contains L1.b3 L1 repetitive element (E -172)
	Unigene Hs#S1170506 qf94g04.x1 Homo sapiens cDNA (E -167)

FIGURE 37I

UNK19	**Genbank nt** **AC005297 Homo sapiens Xp22-149 BAC RPCI11-466O4** **(-perfect match)** **Genbank nr** **2072964 (U93569) putative p150 (E -83)** **207289A reverse transcriptase related protein (E -80)** **Human EST** **AI133053 HA1642 Human fetal liver cDNA library** **(-perfect match)** **Unigene** **Hs#S1540 Human Line-1 repeat mRNA with 2 open reading frames (-perfect match)**
UNK20	**Genbank nt** **AC006480 Homo sapiens clone NH0166O04** **(-perfect match)** **Genbank nr** **Human EST** **Unigene**
UNK21_5'	**Genbank nt** **No significant matches**

FIGURE 37J

	Genbank nr
	Human EST
	Unigene
UNK21_3'	**Genbank nt** **No significant matches**
	Genbank nr
	Human EST
	Unigene
UNK22_5'	**Genbank nt** **D88984S01 Mus musculus Ampd3 gene, exon 1 (E -85)**
	Genbank nr **catalase - Campylobacter jejuni >gi|2120538|pir||S71937 catalase (E -12)**

FIGURE 37K

Human EST
AL042909 DKFZp434J1722_s1 434 (synonym: htes3)
Homo sapiens (E -53)

Unigene
Hs#S1314698 jun1.M13-Control Homo sapiens cDNA (E -41)
Hs#S816313 Homo sapiens mRNA-associated protein mrnp41
(E -24) Hs#S1090518 Homo sapiens p60 katanin mRNA, (E -23)
Hs#S998169 Homo sapiens regulator of G protein signaling
(E -19)

UNK22_3'

Genbank nt
AF082186 Mus musculus proteinase-3 and neutrophil elastase
genes (E -154) May be vector sequence

Genbank nr
E. Coli cloning vector

Human EST
AL044162 DKFZp434P1628_r1 434 (synonym: htes3)
(E -96)

Unigene
Hs#S705542 Uncoupling protein 2 (mitochondrial, proton
carrier) (E -74)

UNK23

Genbank nt
No significant matches

FIGURE 37L

	Genbank nr
	Human EST
	Unigene
UNK24	**Genbank nt** **D85375S2 Human DNA for thyrotropin-releasing hormon receptor** **(E -111)**
	Genbank nr
	Human EST
	Unigene
UNK25_3'	**Genbank nt** **No significant matches**
	Genbank nr
	Human EST

FIGURE 37M

	Unigene
UNK25_5'	**Genbank nt** **AF018071 Homo sapiens D15S1506 ca repeat region (E -21)**
	Genbank nr
	Human EST **AI048523 BSK-M3-RA-III RA-MO-III Homo sapiens (E -20)**
	Unigene
UNK26_5'	**Genbank nt** **AF135422 Homo sapiens GDP-mannose pyrophosphorylas (E -5)** **(weak)**
	Genbank nr
	Human EST
	Unigene

FIGURE 37N

UNK26_3'	**Genbank nt** **No significant matches** **Genbank nr** **Human EST** **Unigene**
UNK27	**Genbank nt** **Genbank nr** **YM3M_CAEEL HYPOTHETICAL 49.0 KD TRP-ASP REPEATS CONTAINING PROTEIN F55F8.5 IN CHROMOSOME I >gi1707049 (U80447) similar to the beta transducin family [Caenorhabditis elegans] (E -20)** **Human EST** **AW058555 wx23d07.x1 NCI_CGAP_Kid11 Homo sapiens (-perfect match)** **Unigene** **Hs#S1266798 tg78g12.x1 Homo sapiens cDNA (-perfect match)**

FIGURE 37O

UNK28

Genbank nt
AC007278 Homo sapiens clone NH0308G20 (E -5)

Genbank nr

Human EST
AA244415 nc07d10.s1 NCI_CGAP_Pr1 Homo sapiens (E -8)

Unigene
Hs#S1120190 qd08f05.x1 Homo sapiens cDNA
E -7)

FIGURE 37P

Table 4: Expressed Sequence Tag (EST) Sequences Producing Significant Alignments with MG-UC1 (SEQ ID NO:8)

		Score (bits)	E Value
gb|AW469670.1|AW469670	hd32f02.x1 Soares_NFL_T_GBC_S1 Homo ...	527	e-148
gb|AW468683.1|AW468683	hd26e04.x1 Soares_NFL_T_GBC_S1 Homo ...	527	e-148
gb|AW118578.1|AW118578	xd94b09.x1 Soares_NFL_T_GBC_S1 Homo ...	527	e-148
gb|AW070300.1|AW070300	xa06b05.x1 Soares_NFL_T_GBC_S1 Homo ...	527	e-148
gb|AI871447.1|AI871447	wl71g10.x1 NCI_CGAP_Brn25 Homo sapie...	527	e-148
gb|AI870604.1|AI870604	wl75d03.x1 NCI_CGAP_Brn25 Homo sapie...	527	e-148
gb|AI809260.1|AI809260	wf69g02.x1 Soares_NFL_T_GBC_S1 Homo ...	527	e-148
gb|AI655835.1|AI655835	tt40e04.x1 NCI_CGAP_GC6 Homo sapiens...	527	e-148
gb|AI187759.1|AI187759	qe12c01.x1 Soares_testis_NHT Homo sa...	527	e-148
gb|AA884358.1|AA884358	al60b05.s1 Soares_NFL_T_GBC_S1 Homo ...	527	e-148.
gb|AA702544.1|AA702544	zf89a09.s1 Soares_pineal_gland_N3HPG...	527	e-148
gb|AA478578.1|AA478578	zv09h06.s1 Soares_NhHMPu_S1 Homo sap...	527	e-148
gb|AA468523.1|AA468523	ne05e08.s1 NCI_CGAP_Co3 Homo sapiens...	527	e-148
gb|AA436169.1|AA436169	zv22c12.s1 Soares_NhHMPu_S1 Homo sap...	527	e-148
emb|Z39728.1|Z39728	HSC1ED042 normalized infant brain cDNA ...	527	e-148
gb|AW511079.1|AW511079	hd38g07.x1 Soares_NFL_T_GBC_S1 Homo ...	519	e-146
gb|AI628680.1|AI628680	ty77h06.x1 NCI_CGAP_Kid11 Homo sapie...	519	e-146
gb|AI590195.1|AI590195	tn49g04.x1 NCI_CGAP_Kid11 Homo sapie...	519	e-146
gb|AI434017.1|AI434017	ti12b09.x1 NCI_CGAP_Pan1 Homo sapien...	519	e-146
gb|AA907552.1|AA907552	om09c10.s1 Soares_NFL_T_GBC_S1 Homo ...	519	e-146
gb|AA757719.1|AA757719	zg43c05.s1 Soares_pineal_gland_N3HPG...	519	e-146
gb|AW467026.1|AW467026	ha07h03.x1 NCI_CGAP_Kid12 Homo sapie...	511	e-143
gb|AI798663.1|AI798663	we91b03.x1 Soares_NFL_T_GBC_S1 Homo ...	511	e-143
gb|AA436296.1|AA436296	zv22c12.r1 Soares_NhHMPu_S1 Homo sap...	511	e-143
gb|T23475.1|T23475	seq3168 1-NIB Homo sapiens cDNA clone Hy...	511	e-143
gb|R89242.1|R89242	yp99e12.s1 Soares fetal liver spleen 1NF...	507	e-142
emb|F01826.1|F01826	HSC0EH062 normalized infant brain cDNA ...	507	e-142
gb|AI914944.1|AI914944	wf61b02.x1 Soares_NFL_T_GBC_S1 Homo ...	494	e-138
gb|H08071.1|H08071	yl86c12.s1 Soares infant brain 1NIB Homo...	492	e-137
gb|AI651919.1|AI651919	wb51b01.x1 NCI_CGAP_GC6 Homo sapiens...	480	e-134
gb|T85591.1|T85591	yd82g11.s1 Soares fetal liver spleen 1NF...	476	e-133
gb|AI659697.1|AI659697	tu25g02.x1 NCI_CGAP_Pr28 Homo sapien...	470	e-131
gb|AI623102.1|AI623102	tu49f09.x1 NCI_CGAP_Pr28 Homo sapien...	470	e-131
gb|R40065.1|R40065	yf70c08.s1 Soares infant brain 1NIB Homo...	464	e-129
gb|AI201359.1|AI201359	qf79h08.x1 Soares_fetal_lung_NbHL19W...	454	e-126
gb|R38728.1|R38728	yd03f05.s1 Soares infant brain 1NIB Homo...	450	e-125
gb|R89246.1|R89246	yp99f12.s1 Soares fetal liver spleen 1NF...	430	e-119
gb|AW271840.1|AW271840	xs11f01.x1 NCI_CGAP_Kid11 Homo sapie...	317	4e-85
gb|AW341763.1|AW341763	he40e09.x1 NCI_CGAP_CML1 Homo sapien...	307	4e-82
gb|AI263919.1|AI263919	qk02b03.x1 NCI_CGAP_Kid3 Homo sapien...	278	3e-73
gb|AW090486.1|AW090486	xc84b02.x1 NCI_CGAP_Brn35 Homo sapie...	250	8e-65
dbj|D25650.1|D25650	HUMGSC3899 Human colon mucosa Homo sapi...	178	2e-43

FIGURE 38

Table 5: Sequences Producing Significant Alignments with UNK5-Derived Amino Acid Consensus Sequence (NIWVSGLSStTrAtDLKNLFsKYGKVvgAKVVTNARSPGArCYGfVTM StseEatkCIaHLHrTELHGkmISVEKaKnEPagKKmSDkndeKSSkekssdvdr)

	Score (bits)	E Value
gb|AAC14666.1| (AC004611) KIAA0138 [Homo sapiens] >gi|14691	166	6e-41
ref|NP_002958.1|| scaffold attachment factor B >gi|2828537|	161	1e-39
gb|AAC18697.1| (L43631) scaffold attachment factor B [Homo	161	1e-39
gb|AAC29479.1| (AF056324) scaffold attachment factor B; SAF	160	3e-39
dbj|BAA91401.1| (AK000867) unnamed protein product [Homo sa	135	8e-32
emb|CAA70700.1| (Y09506) transformer-SR ribonucleoprotein [	66	8e-11
emb|CAB16278.1| (Z99259) putative rna binding ribonucleopro	63	7e-10
sp|Q10422|YDC1_SCHPO HYPOTHETICAL 33.6 KD PROTEIN C25G10.01	63	7e-10
pir||S46286 RNA-binding protein - Wood tobacco >gi|624925|d	59	2e-08
emb|CAB67653.1| (AL132968) RNA-binding protein cp29 protein	57	4e-08
dbj|BAA06519.1| (D31711) cp29 [Arabidopsis thaliana]	57	4e-08
pir||S53490 RNA-binding protein cp29 precursor - Arabidopsi	57	4e-08
gb|AAA81023.1| (U38483) CEBP-1 gene product [Dianthus caryo	57	5e-08
gb|AAF31733.1|AC020579_10 (AC020579) putative RNA-binding p	56	9e-08
sp|P10979|GRPA_MAIZE GLYCINE-RICH RNA-BINDING, ABSCISIC ACI	55	2e-07
gb|AAB71417.1| (U81287) glycine-rich RNA-binding protein Ps	55	2e-07
gb|AAA97963.1| (U55363) coded for by C. elegans cDNA yk102b	55	2e-07
gb|AAA75104.1| (U32310) single-stranded nucleic acid bindin	55	2e-07
pir||S23780 nucleic acid-binding protein - maize >gi|168526	55	2e-07
pir||S18348 RNA-binding protein, 29K - spinach	54	3e-07
emb|CAA84667.1| (Z35601) Similar to the probable RNA bindin	54	3e-07
emb|CAA41023.1| (X57955) 29kD RNA binding protein [Spinacia	54	3e-07
gb|AAB07749.1| (U49482) low temperature-responsive RNA-bind	54	3e-07
sp|P28644|RO28_SPIOL 28 KD RIBONUCLEOPROTEIN, CHLOROPLAST (	54	3e-07
sp|P19682|RO28_NICSY 28 KD RIBONUCLEOPROTEIN, CHLOROPLAST P	54	3e-07
gb|AAC23649.2| (AC004684) putative RNA-binding protein [Ara	54	3e-07
emb|CAA20734.1| (AL031534) RNA binding ribonucleoprotein [S	54	3e-07
sp|P19697|RT19_ARATH 40S RIBOSOMAL PROTEIN S19, MITOCHONDRI	54	3e-07
sp|P49313|RC30_NICPL 30 KD RIBONUCLEOPROTEIN, CHLOROPLAST P	53	4e-07
emb|CAA06469.1| (AJ000286) cp31AHv protein [Hordeum vulgare]	53	4e-07
dbj|BAA22411.1| (D38495) Psi6 protein [Triticum aestivum]	53	4e-07
sp|Q03251|GRP8_ARATH GLYCINE-RICH RNA-BINDING PROTEIN 8 (CC	53	4e-07
pir||S20070 ribonucleoprotein B, 29K - wood tobacco >gi|141	53	4e-07
pir||S20069 ribonucleoprotein A, 29K - wood tobacco >gi|157	53	4e-07

FIGURE 39A

emb|CAA15498.1|	(AL008725) dJ148E22.2 (similar to PAB1) [Ho	53	6e-07
sp|Q99070|GRP2_SORBI	GLYCINE-RICH RNA-BINDING PROTEIN 2 >gi	53	6e-07
gb|AAB71977.1|	(AC002292) putative RNA-binding protein [Ara	53	8e-07
emb|CAA74889.1|	(Y14557) ribonucleoprotein [Pisum sativum]	53	8e-07
emb|CAB36849.1|	(AL033529) glycine-rich RNA-binding protein	52	1e-06
emb|CAA05727.1|	(AJ002892) AtGRP2 [Arabidopsis thaliana]	52	1e-06
pir||S31443	glycine-rich RNA-binding protein (clone A81) -	52	1e-06
sp|P49314|RC31_NICPL	31 KD RIBONUCLEOPROTEIN, CHLOROPLAST P	52	1e-06
pir||S50765	RNA-binding protein - common ice plant >gi|3886	52	1e-06
emb|CAA05729.1|	(AJ002894) CsGRP2 [Oryza sativa]	52	1e-06
gb|AAB88615.1|	(AF034945) glycine-rich RNA binding protein	52	1e-06
pir||S20946	glycine-rich protein - maize >gi|22293|emb|CAA4	52	1e-06
emb|CAB71336.1|	(AL250661) putative Bci-5 protein [Hordeum	52	1e-06
pir||S53050	RNA binding protein - barley >gi|728594|emb|CAA	52	2e-06
sp|P19683|RC31_NICSY	31 KD RIBONUCLEOPROTEIN, CHLOROPLAST P	52	2e-06
pir||S49463	chloroplast RNA binding protein - kidney bean >	51	2e-06
emb|CAA11893.1|	(AJ224324) cp31BHv [Hordeum vulgare]	51	3e-06
pir||S49030	RNA-binding protein rnpD - Arabidopsis thaliana	51	3e-06
sp|Q04836|RC31_ARATH	31 KD RIBONUCLEOPROTEIN, CHLOROPLAST P	51	3e-06
dbj|BAA06521.1|	(D31713) cp31 [Arabidopsis thaliana]	51	3e-06
pir||S53492	RNA-binding protein cp31 precursor - Arabidopsi	51	3e-06
gb|AAA18380.1|	(U08467) RNA-binding protein 3 [Arabidopsis	51	3e-06
pir||S20940	DNA-binding protein - Arabidopsis thaliana	51	3e-06
emb|CAA73034.1|	(Y12424) SGRP-1 [Solanum commersonii]	51	3e-06
gb|AAA18379.1|	(U08467) RNA-binding protein 2 [Arabidopsis	51	3e-06
gb|AAB07067.1|	(U59476) RNA-binding protein lark [Drosophil	50	4e-06
emb|CAB71228.1|	(AL138658) hypothetical protein L3277.06 [L	50	4e-06
sp|Q05966|GR10_ERAMA	GLYCINE-RICH RNA-BINDING PROTEIN 10 >g	50	5e-06
gb|AAC98043.1|	(AC005896) putative RNA-binding protein [Ara	50	5e-06
gb|AAC61786.1|	(AF036239) glycine-rich RNA-binding protein	50	7e-06
emb|CAB63054.1|	(AL049748) dJ41P2.2 (RNA binding motif prot	50	7e-06
dbj|BAA91472.1|	(AK001027) unnamed protein product [Homo sa	50	7e-06
emb|CAA15842.1|	(AL009266) hypothetical protein [Homo sapiens]	50	7e-06
emb|CAB63055.1|	(AL049748) dJ41P2.2 (supported by GENSCAN)	50	7e-06
gb|AAB66885.1|	(AF010580) glycine-rich protein [Oryza sativa]	49	9e-06
gb|AAB65412.1|	(AF011331) glycine-rich protein [Oryza sativa]	49	9e-06
gb|AAB63581.1|	(AF009003) glycine-rich RNA binding protein	49	9e-06
pir||S40774	ribonucleoprotein - African clawed frog >gi|214	49	1e-05
gb|AAF06329.1|AF191305_1	(AF191305) glycine-rich RNA bindin	49	1e-05
dbj|BAA98978.1|	(AB025351) BFCIRP [Rana catesbeiana]	49	1e-05
gb|AAC98546.1|	(U18366) stage specific activator protein; S	48	1e-05
gb|AAB63589.1|	(AF009411) glycine-rich RNA-binding protein	48	1e-05
ref|NP_032665.1|	Musashi-1 homolog (Drosophila) >gi|143485	48	1e-05

FIGURE 39B

emb|CAB36850.1|	(AL035529) RNA-binding protein like [Arabid	48	1e-05
prf||2106321A	stage-specific activator protein [Strongyloca	48	1e-05
ref|NP_002433.1||	Musashi (Drosophila) homolog 1 >gi|276959	48	2e-05
gb|AAC53171.1|	(U89506) Mlark [Mus musculus]	48	2e-05
gb|AAC65342.1|	(AE001215) RNA-binding protein, putative [Tr	48	2e-05
emb|CAA64070.1|	(X94344) Neosin [Mus musculus]	48	2e-05
ref|NP_002887.1||	RNA binding motif protein 4 >gi|2078529|g	48	2e-05
gb|AAC41383.1|	(U71299) RNA-binding protein AxRNBP [Ambysto	48	2e-05
emb|CAB43861.1|	(AL078465) hnRNP-like protein [Arabidopsis	48	3e-05
ref|NP_001271.1||	cold inducible RNA-binding protein >gi|59	48	3e-05
gb|AAD48471.1|AF169205_1	(AF169205) glycine-rich RNA-bindin	48	3e-05
emb|CAA05399.1|	(AJ002414) hnRNP-like protein [Arabidopsis	48	3e-05
sp|P49310|GRP1_SINAL	GLYCINE-RICH RNA-BINDING PROTEIN GRP1A	48	3e-05
pir||JC4817	RNA-binding protein RZ-1 - wood tobacco >gi|139	48	3e-05
gb|AAB63640.1|	(AC001645) poly(A)-binding protein isolog [A	48	3e-05
gb|AAA83161.1|	(U41531) similar to C. elegans protein R74.5	48	3e-05
emb|CAA05728.1|	(AJ002893) OsGRP1 [Oryza sativa]	48	3e-05
gb|AA.67723.1|	(U14946) ribonucleoprotein [Caenorhabditis e	48	3e-05
dbj|BAA91479.1|	(AK001049) unnamed protein product [Homo sa	47	3e-05
emb|CAA76346.1|	(Y16672) putative arginine/serine-rich spli	47	3e-05
gb|AAF31404.1|AF200323_1	(AF200323) putative glycine-rich R	47	3e-05
dbj|BAA22083.1|	(D28862) RNA binding protein [Nicotiana syl	47	3e-05
pir||S54255	probable glycine rich RNA binding protein - pot	47	3e-05

FIGURE 39C

SEQUENCE LISTING

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Hernnstadt, Corinna
Miller, Scott W.
Davis, Robert E.

<120> DIFFERENTIAL EXPRESSION OF ORGANELLAR GENE PRODUCTS

<130> 660088.419PC

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gtcttgcatt tggcagaaac tcacaggctg gcagaacagt gaaaaaggtt cacactggaa 180

aagagagaag gcttcagggg tgcctgattg gaggtagttg gcgtangaaa gctggaagtg 240
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tttcagtaat ctgtaaatat ctttatattt gagaaaattt gtgagacatc tttgtgtaaa 360
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<213> Homo sapien

<400> 13
ggattcagac taaaaggaag agatgtg 27

<210> 14
<211> 25
<212> DNA
<213> Homo sapien

<400> 14
aaatcttcct ctaacatggc caact 25

<210> 15
<211> 19
<212> DNA
<213> Homo sapien

<400> 15
cgccaagtgg atggatttg 19

<210> 16
<211> 23
<212> DNA
<213> Homo sapien

<400> 16
ggaggagctt tgatctcaca tga 23

<210> 17
<211> 21
<212> DNA
<213> Homo sapien

<400> 17
gattcagagc ttgccctagc a 21

<210> 18
<211> 24
<212> DNA
<213> Homo sapien

<400> 18
ccagtgtgaa ccttttcac tgtt 24

<210> 19
<211> 29
<212> DNA
<213> Homo sapien

<400> 19
agaaaatttg tgagacatct ttgtgtaaa 29

<210> 20
<211> 28
<212> DNA
<213> Homo sapien

<400> 20
ctggttataa gttatatcct cgcagcta 28

<210> 21
<211> 29
<212> DNA
<213> Homo sapien

<400> 21
gagctgatac tattcccact gaaactatt 29

<210> 22
<211> 27
<212> DNA
<213> Homo sapien

<400> 22
tgtctctacc aggttttggt attagga 27

<210> 23
<211> 394
<212> DNA
<213> Homo sapien

<400> 23
cgactccaag ataggcagat tgtggagaaa taaatatttc cctagtcatt gtgattacat 60
tcctaatgga ccttctctgg tgctgatact gaaatagtac aaaaagttgt cagtaccttt 120
caattctgtt ggtcaaaaat atgtttttcc ttttttgtg tgtgtgtttt ttttttcctt 180
taaaatgaac atatacttcc aacatagaag ttgtaacctt tatatttaac caagtttcca 240
gttgaagcca gtttggggtg tgcatgtgtg tgcatgtgtc tatatgcgtg tgtgtgtata 300
tacacacaca ccaattatat atatagtatg catgtgtgta tgtacataca gagaattttt 360
gagctggggc ctttttagca gtaaaaaaaa aaaa 394

<210> 24
<211> 510
<212> DNA
<213> Homo sapien

<400> 24
gctagcatgg ccaacatggt gaaaccccgt ctctacaaaa gaaaaaaata caaaaattag 60
ctgggtgttg tggtgtatgc ctgtaatccc aactatttgg gtggctgagg cacgagagtc 120
gcttggactt ggggggcgga ggttgcagtg agctgagatc gtgccactgc actccagcct 180
gggtgacaga ctgagacagt ctcaaaaaaa aaaaaaagaa aataatggat ttgcagagac 240
ttgctattta gatttcagac atctgttaac taaaacacat gtgtaggctt ttgttactta 300
tttcagtaat ctgtaaatat ctttatattt gagaaaattt gtgagacatc tttgtgtaaa 360
ttataacttg aagaacctct cttacaagca ggcatattgg taagtagctg cgaggatata 420
acttataacc agattgaagt gtataattat aatatgttat tattctgggg ttctataaaa 480
aataaaatct ttgaatctaa aaaaaaaaaa 510

<210> 25
<211> 622
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(622)
<223> n = A,T,C or G

<400> 25
gctagcagac gacaagaaat aaccaagatc agagctgaac tgaaggagat tgagacacaa 60
aaaaatttaa aagatcaatg aatccagaaa ctcattcttt gaaaaaactc agtaaaatag 120
actgctagct agactaataa aaaagaaaag agagaagatt caaataaaca cnatcagaag 180
taataagggg gataatacca ctgaccccac agaactacaa acaaccatta gaggagtcta 240
tatntataaa ctggaaaatg tagaagaact ggatacattn ctggacacgt acacctccca 300
agactgacca ggaagaattg atccctgata gactaattca tggaattctg gaaattgagt 360
cagtaataaa tagcttacca accagaaaca agcccaggat cagacagatt cacagctaaa 420
ttctaccaga tgtacaaaga agagctgata ctattcccac tgaaactatt ccaaaaattg 480
aggaggaggg actcttctct aacatgctat gaggccagca tcatcctaat accaaaacct 540
ggtagagaca caacaaaaaa aaataaaact tcaggccaat atccttgatg aacattgacg 600
caaaaatcct aaaaaaaaaa aa 622

<210> 26
<211> 537
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(537)
<223> n = A,T,C or G

<400> 26
gaccattgca ttataatgga agagggacca tataaagagc cagaattact gggtgctaat 60
tctaaccatg cctgggctgc cctcatctct atgactgttc ttcctgagtt ctttgcaggt 120
taatttcctt tgtgtagtca taaaatgata aattcgccct gaataacagt ctaggctcta 180
ctctaacccc acactatctt ctgagtaggc ttacaaagcc taannnttac aaagcngagn 240
ngnatcnagc tgtcaaaagt gattagaaat tttaaatgat cantccagcc tttaatttgg 300
tatatgcacc atattaagtc atttaagtga gtcagtaaat gtggcttgta atataagaat 360
gacagtaata tctatatgtg tatattcttt gattgtcagt gatgcatcaa tttaccaaaa 420
acagcagata acaacttaaa atatacttta ctattttcaa attgcagttt gattaagtgc 480
aattgcaatt gtacttaatt gaaaaaaatc aagttttata tgaacaaaaa aaaaaaa 537

<210> 27
<211> 691
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(691)
<223> n = A,T,C or G

<400> 27

gaccattgca cagagcccgg agaaggaaag caaggattat gagatgaatg cgaaccataa 60
agatggtaag aaggaagact gcgtgaaggg tgaccctgtc gagaaggaag ccagagaaag 120
ttctaagaga gcagaatctg gagacaaaga aaaggatact ttgaagaaag ggccctcgtc 180
tactggggcc tctggtcaag caaagagctc ttcaaaggaa tctaaagaca gcaagacatc 240
atctaaagat gacaaaggaa gtacaagtng tactagtggt agcagtggaa gctcaactaa 300
aaatatctgg gttagtgaac tttcatctaa taccaaagct gctgatttga agaactcttt 360
ggcaaatatg gaaaggttct gagtgcaaaa gtagttacaa atgctcgaag tcctggggca 420
aaatgctatg gcattgtaac tatgtcttca agcacagagg tgtccaggtg tattgcacat 480
cttcatcgca ctgagctgca tggacagctg atttctgttg aaaaagtaaa aggtgatccc 540
tctaagaaag aaacgaagaa agaaaatgat gaaaagagta gttcaagaag tcctggagat 600
aaaaaaaata cgagtgatag aagtagcaag acacaagcct ctgtcaaaaa agaagagaaa 660
agatcgtctg agaaatctca aaaaaaaaaa a 691

<210> 28
<211> 392
<212> DNA
<213> Homo sapien

<400> 28
cgactccaag ccctgactct ttgctgcgcc tgagacaaaa taaactttcc ataaaagact 60
gagaatagaa tacaaagtag tatacatagc tataaccaat aaacaaatta tgtctttaaa 120
aatatcccaa atgtgtgcag aaaaaaacat taacagtgac cgtctttgag tagtagatat 180
gaccaatatt attctctttg ctataaatag tattccaaat tttaataata cacttttttaa 240
atatttgtat acatactttt atatttcact atactgtgtt gaaaagtata tattgtaata 300
agctatttta tacatgaaag aaaaaaattt ttgcatcata agttgtatat attataataa 360
actatttta agttaccttg aaaaaaaaaa aa 392

<210> 29
<211> 567
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(567)
<223> n = A,T,C or G

<400> 29
gctagcatgg caagcgccca taaatgccag taactgtgga tgctgccaga agtcagctgt 60
ttccagggac acagtgtagc tgggttgcat tttacagttt aatgtaactc gggtcgtctt 120
ctgtgggagt aaactcatgt ttttgtgact gttttatggg tttgtccctc atattggagc 180
ttagtctaag ctgcgcctca gactcctgtg tctgtcatgc tgggagcctt tggagaacgg 240
tccgtttgtc caacgtccag tttgctgagc atttttaaat ccaactctgc acttacacct 300
ggccaggcag gaatgctccc agaatgggtc ggcagtgtag aaagagatcc tgagaagtgg 360
gtttctntct tttggtcaaa acttacctgt tttgcatgaa catttaaaag tctgtcttga 420
tcccaatttg gaacaatatg cctcaaaacc ataaaggttg tatttaccag cctgatgttg 480
atttgactaa tgttaatttg cgagagatga atattagtat cttttaaata aaaaatgcct 540
gcctatttca ctatcaaaaa aaaaaaa 567

<210> 30
<211> 567
<212> DNA
<213> Homo sapien

<400> 30

gctagcatgg gtgatagagt gagatcgtct ccaaactctc ctttctgaaa ttttacttag 60
ctaaattttt tcctaattcc tcctcagtat ttccacttga ttcccccaca gaatgtaatt 120
gtaatgtatt tattagtatc tttgaacatc ttttattatt tgcctatcat acttctctac 180
aacaaaatat atgtaagtta ataaaaatat ttcctgtgta catgatattg tcttaaattt 240
cttctatatt tagttattac attacattta ttattaggac atggcaattg aaggagcata 300
aatatacttt gttttgccaa actagtatga aacatttaaa aatgaaattt tactgaatat 360
atgcattagt gagaaggatg gtccttatta atatagttgt aggtgaatat taagctagaa 420
tggtagtgtt cattaattct ctcttcctat tttctatttt tatatatgtg aattctaaaa 480
aaccttattt acataatgtt tttagtgcac atggaagttt ttgataactt tttaaattga 540
atttcttctg aattataagt caaaaaa 567

<210> 31
<211> 460
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(460)
<223> n = A,T,C or G

<400> 31
gntagcagac acattttcaa agggtcatat tcttggcttg ttggtaatca gaatcgggca 60
ggagaagtgg ggtggatgca gaccagctga ccacactggc accaccagca gtttcagttt 120
cgtcttgatt gtaaagagga aatatctaat cttaaaactc attaggggcc tggcgcagtg 180
gctcatacct gtattcccaa cactttggga ggccgaggca ggcagatcac ccgaggtcag 240
gattttgaga ccagcctggc caacatggtg aaaccccatc tctactaaaa atacaaaact 300
tagctaggcg tgatggcagg cacctctaat cccagttact tgggaggctg aggcaggaga 360
atcacttgaa cccggaaggc agacgttgca gtgagccaag atcgtgccac tgcactccag 420
cctgggcaac tagagcaaga ctccatctaa aaaaaaaaaa 460

<210> 32
<211> 258
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(258)
<223> n = A,T,C or G

<400> 32
gaccattgca cagagcccgg agaaggaaag caaggattat gagatgaatg cgaaccataa 60
agatggtaag aaggangact gcgtgaaggg tgaccctgtc gagaaggaag ccagagaaag 120
ttctaagaaa gcagaatctg gagacaaaga aaaggatact ttgaagaaag ggccctcgtc 180
tactggggcc tctggtcaag caaagagctc ttcaaaggaa tctaaagaca gcaagacatc 240
atctaaagat gacaaagg 258

<210> 33
<211> 259
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature

<222> (1)...(259)
<223> n = A,T,C or G

<400> 33
ggcattgtaa ctatgtcttc aagcacagag gtgtccaggt gtattgcaca tcttcatcgc 60
actgagctgc atggacagct gatttctgtt gaaaaagtaa aaggtgatcc ctctaagaaa 120
gaaatgaaga aagaaaanga ngaaaagagt agttcaagaa gttctggaga taaaaaaata 180
cgaatgatag aagtagcaag acacaagcct ctgtcaaaaa agaagagaaa agatcgtntg 240
agaaatcaaa aaaaaaaaa 259

<210> 34
<211> 696
<212> DNA
<213> Homo sapien

<400> 34
ggaccattgc attaaaatgt tttggatacc tgtttgaata acattgcctt aatgttaata 60
aatccataat ggtcacacag gcaggggtgg tgtgtgaatc accctggaag ggatgttcat 120
taatcagtta cttggggctt ttttctttat tcattccctc ctagggtttg tacctgtgag 180
gaagcagcct accttctttg cagccatcta gcatctatat tctagaatca tttttcccta 240
tgatggtcaa atccagatta tctacacaga agaataaaat aacctgagta atccaaagtg 300
agtcataagt ttttaaaagt ctgggccagg cacagtgtct catgcctgta atcccagcat 360
tttaggaggc ccaggaggga ggatcacttg agcttaggag ctcgagacca gcctgagcaa 420
catagtgaga ccccatttct accaaaaata gttttaaaaa tagccagaca tggtggtgca 480
tccctgtggt cccaggcagt tggtggctga ggtgggagga tcctttgaac ccaggaggtt 540
gaggttggag tgagctatga tggatcacac cactgcactc cagcctgggc aaccgagtga 600
aacccttttct caaaaatatg cattgtcctt tggaatatgt tctgtattcg aacatggatg 660
tagctaatgt ttgattttaa ttacaaaaaa aaaaaa 696

<210> 35
<211> 393
<212> DNA
<213> Homo sapien

<400> 35
gaccattgca aaatactgta gaagaactgt ttagcttgct tcatttcttg gaaccgtcac 60
aatttccctc agaatcagag tttctcaagg actttgggga tctcaagaca gaggaacagg 120
ttcaaaagct acaggccatt cttaagccaa tgatgctgag aagactcaaa gaggatgttg 180
aaaaaaactt ggcacccaaa caggaaacaa ttattgaagt agagctgact aatatccaga 240
agaaatacta tcgggctatt ttggagaaga atttctcctt cctttccaaa ggggcaggtc 300
ataccaacat gcctaatcta cttaacacaa tgatggagtt gcgcaagtgc tgcaaccacc 360
catatctcat caatggtgct gaaaaaaaaa aaa 393

<210> 36
<211> 253
<212> DNA
<213> Homo sapien

<400> 36
gaccattgca cagtaaatcc attgtaggct ttctttatgg gtggcggggg aatctctaaa 60
ggtcaggagt ccagattgct tcaaataaac atccagaatc tcagatgctt ttttgaaaca 120
agcccaagtt tatctgaacc tctttctctg gtttggaaat caggctgaaa atgtcacaga 180
aacagatttt cttgtgagat ctcagaatgt tgtggtttaa gtaaagtaat aaacaaagtc 240
gaaaaaaaaa aaa 253

<210> 37
<211> 307
<212> DNA
<213> Homo sapien

<400> 37
ggccattgca taaaagtaac tttacaagaa tattagcact aaataattcc taacatcttg 60
gagacaattt tcaaaataat gcgtttgttt actcattcaa gatgtattta ctgagctacc 120
actgttatat gccaagtgat gttctaggtc ctagacatgt agcaaaaacc aaactgaaaa 180
aaaaattaac tcttgtagat tttcaaagca actatagcag caagaggaag agacgaacac 240
cgaaaaaaaa aaagccctat agtgagtcgt attaagccga attctgcaga tatccatcac 300
actggcg 307

<210> 38
<211> 481
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(481)
<223> n = A,T,C or G

<400> 38
gaccattgca atgaatcccc aataattgca gaactaaact catttataaa gctaaaataa 60
ccggatatat acatagcatg acatttcttt gtgctttggc ttacttgttt aaaaaaaaaa 120
aaaactaatc caacctgtta gatttngcag gtgaagtcag cagcttaaaa atgtctttcc 180
cagatttcaa tgatttttt ccccctacct cccaaaatct gagactgtta aaacatttt 240
ctcctatgaa cactgctcag acctgcttcg acatgccata ggagtggcgt gcacatctct 300
ctctcttcca gcaggaggag cccgtgagca cgcacagctg ccctgtctgc tcacccgaag 360
gcaccgggct cacctggacc tcccaggaaa gggagaaaga gcctccagaa actgctctgt 420
gtttagaaag gaatatcttt aagaatccaa gtttttcatt tccacaaatt tcctatatcc 480
a 481

<210> 39
<211> 450
<212> DNA
<213> Homo sapien

<400> 39
gatttgtttg gacaatgtag ttgggaagaa ctaagattct aatctgtgaa gaaccttata 60
gggccttcta aaacataaga gtttcctttg ttgcttcaaa tatttgaaca ttatgttaaa 120
gatcaagtat taattttagt tgtactctag aaagctaaag tgccacattc ggggctattt 180
ttatgattca gcaatctttt ctaaattgtg tagcatgtgt atgagactat ttatacccaa 240
ggatatgaag gaatataagt gactacaagg ctctaataag ccacggtggc aggaggttca 300
agcggttctg ttcactaaat ttttctcctg taagctttga atggaaactt ctgtatcaca 360
tgatgtgttt cacttatgct gttgtgtata tacctaatat ttctattttt gattttattt 420
taatacacct cgtccaataa aaaaaaaaaa 450

<210> 40
<211> 420
<212> DNA
<213> Homo sapien

<220>

<221> misc_feature
<222> (1)...(420)
<223> n = A,T,C or G

<400> 40
gctagcagac ccacttaagg atgaattaaa ccttgctgat tctgaagtgg ataaccaaaa 60
acgagggaaa cgacnttatg aagaaaaaca aaaagaacac ttggatacct taaataaaaa 120
gaaacgagaa ctggatatga aagagaaaga actagaggag aaaatgtcac aagcaagaca 180
aatctgccca gagcgtatag aagtagaaaa atctgcatca attctggaca aagaaattaa 240
tcgattaagg cagaagatac aggcagaaca tgctagtcat ggagatcgag aggaaataat 300
gaggcagtac caagaagcaa gagagaccta tcttgatctg gatagtaaag tgaggacttt 360
aaaaaagttt attaaattac tgggagaaat catggagccc agattccaga catatcaacc 420

<210> 41
<211> 507
<212> DNA
<213> Homo sapien

<400> 41
gaccacaaga tgaaattcta agtatatcag ttcagcctgg agaaggaaat aaagctgctt 60
tcaatgacat gagagccttg tctggaggtg aacgttcttt ctccacagtg tgttttattc 120
tttccctgtg gtccatcgca gaatctcctt tcagatgcct ggatgaattt gatgtctaca 180
tggatatggt taataggaga attgccatgg acttgatact gaagatggca gattcccagc 240
gttttagaca gtttatcttg ctcacacctc aaagcatgag ttcacttcca tccagtaaac 300
tgataagaat tctccgaatg tctgatcctg aaagaggaca aactacattg cctttcagac 360
ctgtgactca agaagaagat gatgaccaaa ggtgatttgt aacttaacat gccttgtcct 420
gatgttgaag gatttgtgaa gggaaaaaaa attctgaact ctttgatata ataaaatgag 480
actggaggca ttctcaaaaa aaaaaaa 507

<210> 42
<211> 513
<212> DNA
<213> Homo sapien

<400> 42
gctagcagac tatcattaac caaataaatt atgggatttt gtcttaatta tatacatata 60
catatacaca cacatacaca tacacataca tgtgtatata ttccctaaaa cttaataaag 120
ctcaaataat aaaatcagat ttcttaagta ttccaattcc ctttaaaatg taaatcagat 180
tttataattc ttttgttcaa aactgtccat tggctcccat ttcacttaaa tcaaaagcta 240
gtttttacaa taagctaagg tagcaaacat tattatctat ttacttatga gttacttatg 300
taactcagca tccaataaca ctgtaggtgc tcaataaaat agttgctgaa tggataactt 360
tcactatttg gatgagatcc aacagaaaag aatactctta gcttgacaaa caatggtaaa 420
cagaagttaa cattagaaca ctagatcctt gctcactaaa atcagacata attatatgtt 480
tgtgtgtgtg tgtaaatata aacgtatata tgt 513

<210> 43
<211> 489
<212> DNA
<213> Homo sapien

<400> 43
tacatatttg aattaaatga aatatatcag aatttgtggt aacaacggat taaagcttag 60
ttcagaaaag aagaaagttt tcaaatcagc gatataataa tttccaaact taagaaacta 120
gaagagcaaa ttgaaccaaa gcaggcagaa tggaagaaag aataagataa gaaaatcaat 180
gaaattaaaa gcaacagaaa ctaaggccag gtgcagtggc tcatgcctgt aatcccaaca 240

cttcgggagg ccgaggtggg caggtcacgt gaggtcagga gtttgagacc agcctaacca 300
tcatggcaaa accatctcta ctaaaaatac aaaaataagc tgggcatggt ggcaggcacc 360
agtaatccca gctactcggg agactgaggc agaagaatca ctctgggagg cagaggctgt 420
agtgagctga gattgccact gcactctagc ctgggctaca gagtgagact ccatctcaaa 480
aaaaaaaaa 489

<210> 44
<211> 505
<212> DNA
<213> Homo sapien

<400> 44
ggtttttga aaggatcaac aaaattgata gatctctagc aagactaata agaaaagaga 60
gaagaatcaa gtggatgcaa taaaaaatga taaaggggat atcaccactg attccataga 120
agtacaaact accatcagag aatactacaa acacctctac acaaataaac tagaaaatct 180
agaagaaatg gataaattcc tggacacata cacccaccca agactaaacc aggaagaagt 240
tgaatctctg aatagaccag taacaggctc tgaaatggag gcaataatta atagcttacc 300
aaccaaaaaa agtccaggac cagatggaat cacagctgaa ttctgtcaga ggtacaaaaa 360
ggagctggta ccattccttc tgaaactatt ccaatcaata ggaaaagagg gaatcctccc 420
taactcattt tatgaggcca gcaccatcct gataccaaag cctggcagag acgcgacaaa 480
aagaatttta caccaaaaaa aaaaa 505

<210> 45
<211> 506
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(506)
<223> n = A,T,C or G

<400> 45
gctagcagac ggcgagaaat aactaaaatc agagcacaac tgaaggaaat agagacacaa 60
aaaacccttc aaaaaattaa ggaatccagg agctggtttt ttgaaaggat caacaaaatt 120
gatagaccac tagcaagact aataaagaag aaaagagaga agantcaaat agacgcaata 180
aaaaatgata aaggggaaat caccaccaat cccacagaaa tacaaactac catcagagaa 240
tactacaaac acctctatgc aaataaacta gaaaatctag aagaaatgga taaattcctc 300
gacacataca ccctcccaag actaaaccag gaagaagttg aatctctgaa tagtccaata 360
acaggctctg aaattgtggc aataatcaat agcttaccaa ccaaaaagag tccaggacca 420
gatggattca cagccaaatt ctaccagang tttaaggaag aactggtacc attccttctg 480
aaactactct aatcnataga aaaaga 506

<210> 46
<211> 488
<212> DNA
<213> Homo sapien

<400> 46
gctagcagac cacaaaggac gttgatccct gagggaggtg aatcctatga aggctctggc 60
ttaagcccgt gaaggtttct tagcagtggc acaggaaggg caactaactc aagggaggca 120
aactccatga attgaggaaa cagagctaaa gatatgggat aataaagtag ctagagctta 180
caggacagag ttcaggagag agcagatgca cagacaacaa tctcttgaaa tctgcagaga 240
gtctcagaga tctggatatg tgcatgagga agctacccaa ggctgaggaa agaagcatcg 300
gaaataatta tacggggaac agtacctggc accttcctag ggctggaaat aggccttttc 360

tcaccagtca gaatggaaaa tctcttaatt catatgcaat taggtagaac ctcagtagtg 420
agaaatgagg tagactatgc actgctctgg tctctcctag ctaacatttt aatcccaaaa 480
aaaaaaaa 488

<210> 47
<211> 267
<212> DNA
<213> Homo sapien

<400> 47
atggtagtct catcacacac tacaattacc ttcccttaca ttactaattt gaagcataat 60
taacacaagc ctcacatact tggtaaagtt tgctatgtta tagttaaagt ctgtcttcac 120
agatcactac gtttgtgact cattgcgagt tcaataatca aagttcatga actcgaggtg 180
attgatacac agtgtcctca tcagtgaacc tggtgttaat gtagtatttg tccagaaagt 240
tattgtgagg actgtataaa cccttgc 267

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tttggaaagg gcctggcaca taaaaaagct attaatatta aatactatta ttagtatcaa 180
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<210> 51
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<212> DNA
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catcccntcc actnnnctaa ctnngccccc atacaccctc acccccatca agcccttgcc 180
caggacagac nnggacagct ctcccctact cagacacgaa aaaaaaaaaa gccctatagn 240
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<211> 85
<212> DNA
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cagccctagt ttactgtatg tgtatttgga aaggaatcca cagatgattc tgacatgtga 420
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agtcttagaa tattaagtgt tacttttttgt tggaaatggc ttcttttgtc tttattaaag 540
ttaggaatgt gttttctgaa aagcttactt tttgatatta atttccattt ttaaagaaat 600
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20 25 30
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35 40 45
Ser Thr Ala Glu Glu Ala Thr Lys Cys Ile Asn His Leu His Lys Thr
50 55 60
Glu Leu His Gly Lys Met Ile Ser Val Glu Lys Ala Lys Asn Glu Pro
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<213> Homo sapien

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35 40 45
Ser Ser Ser Thr Glu Val Ser Arg Cys Ile Ala His Leu His Arg Thr
50 55 60
Glu Leu His Gly Gln Leu Ile Ser Val Glu Lys Val Lys Gly Asp Pro
65 70 75 80
Ser Lys Lys Glu Met Lys Lys Glu Asn Asp Glu Lys Ser Ser Ser Arg
85 90 95
Ser Ser Gly Asp Lys Lys Asn Thr Ser Asp Arg Ser Ser Lys Thr Gln
100 105 110
Ala Ser Val Lys
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<210> 62
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<212> PRT
<213> Homo sapien

<400> 62
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20 25 30
Thr Asn Ala Arg Ser Pro Gly Ala Arg Cys Tyr Gly Phe Val Thr Met
35 40 45
Ser Thr Ser Asp Glu Ala Thr Lys Cys Ile Ser His Leu His Arg Thr
50 55 60
Glu Leu His Gly Arg Met Ile Ser Val Glu Lys Ala Lys Asn Glu Pro
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Ala Gly Lys Lys Leu Ser Asp Arg Lys Glu Cys Glu Val Lys Lys Glu
85 90 95
Lys Leu Ser Ser Val Asp Arg His His Ser
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<210> 63
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<212> PRT
<213> Unknown

<220>
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<400> 63

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            20                  25                  30
Thr Asn Ala Arg Ser Pro Gly Ala Arg Cys Tyr Gly Phe Val Thr Met
        35                  40                  45
Ser Thr Ser Glu Glu Ala Thr Lys Cys Ile Ala His Leu His Arg Thr
    50                  55                  60
Glu Leu His Gly Lys Met Ile Ser Val Glu Lys Ala Lys Asn Glu Pro
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Ala Gly Lys Lys Met Ser Asp Lys Asn Asp Glu Lys Ser Ser Lys Glu
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<400> 64

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aggaagattt gttagccttg aaatctcctt caaaagaaaa tattgcatct gtcttagaaa     180
attaccatac agagtctaag attgatcgag acaagtcttt tatacttgag gaacacatgg     240
acaaaataaa cagttgtttt tcagccaata ctgtggaaga aattattgaa aacttacagc     300
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tgtgagatca aagctcctcc aaagcctgtt caagctctaa gcgattctca aatgttacca     180
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gtagttaatg tatttattaa tgcttgactt ttaaaaycct gggcataaat agtgcagagc     300
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<400> 66

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gtagttaatg tatttattaa tgcttgactt ttaaaatcct gggcataaat agtgcagagc     300
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INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 00/07311

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C12N15/10 C12N5/10 C12Q1/68 C07K14/47
G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, MEDLINE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 21944 A (SMITHKLINE BEECHAM CORP ;ROSENBERG MARTIN (US); DEBOUCK CHRISTINE) 17 August 1995 (1995-08-17) the whole document ---	1,20,25, 29-31, 33,34
X	WO 98 21321 A (QBI ENTERPRISES LTD ;EINAT PAZ (IL); SKALITER RAMI (IL); GROSMAN Z) 22 May 1998 (1998-05-22) the whole document ---	1-3,6, 20,22-25
X	WO 97 30065 A (MILLENNIUM PHARMACEUTICALS INC) 21 August 1997 (1997-08-21) the whole document --- -/--	1-3,6, 20, 22-26, 29-31, 33,34

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 August 2000	18/08/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kania, T

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INTERNATIONAL SEARCH REPORT

Inte .onal Application No
PCT/US 00/07311

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	CHOI D -K ET AL: "Fluorescent differential display analysis of gene expression in apoptotic neuroblastoma cells" GENE.NL.ELSEVIER BIOMEDICAL PRESS. AMSTERDAM, vol. 223, no. 1-2, 26 November 1998 (1998-11-26), pages 21-31, XP004153574 ISSN: 0378-1119 the whole document	1-3,6, 20,22, 23,25,29
X	SWALLA, BILLIE J.: "Strategies for cloning developmental genes using closely related species" MOL. ZOOL., 'MOL. APPROACHES ZOOL. EVOL. SYMP.! (1996), MEETING DATE 1995 197-208. EDITOR(S): FERRARIS, JOAN D.;PALUMBI, STEPHEN R. PUBLISHER: WILEY-LISS, NEW YORK, N. Y., XP002143746 abstract	1,28
X	MATSUMURA F ET AL: "Differential expression of tropomyosin forms in the microfilaments isolated from normal and transformed rat cultured cells." JOURNAL OF BIOLOGICAL CHEMISTRY, (1983 NOV 25) 258 (22) 13954-64. , XP002143747 abstract	1-3,6, 20,26,29
A	WO 95 26973 A (APPLIED GENETICS INC ;HERRNSTADT CORINNA (US); PARKER WILLIAM DAVI) 12 October 1995 (1995-10-12) cited in the application the whole document	1-35
A	ITO SAYAKA ET AL: "Functional integrity of mitochondrial genomes in human platelets and autopsied brain tissues from elderly patients with Alzheimer's disease." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 96, no. 5, 2 March 1999 (1999-03-02), pages 2099-2103, XP002143748 March 2, 1999 ISSN: 0027-8424 the whole document	1-35

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INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 00/07311

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	SWERDLOW R H ET AL: "Cybrids in Alzheimer's disease: A cellular model of the disease?" NEUROLOGY, vol. 49, no. 4, 1997, pages 918-925, XP000929551 ISSN: 0028-3878 the whole document	1-35
A	WO 98 17826 A (MITOKOR) 30 April 1998 (1998-04-30) the whole document	1-35

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte onal Application No
PCT/US 00/07311

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9521944 A	17-08-1995	EP 0743989 A	27-11-1996
		JP 9508800 T	09-09-1997
WO 9821321 A	22-05-1998	US 6013437 A	11-01-2000
		AU 5258098 A	03-06-1998
		EP 0942969 A	22-09-1999
WO 9730065 A	21-08-1997	AU 2124397 A	02-09-1997
		CA 2247246 A	21-08-1997
		EP 0904277 A	31-03-1999
		US 6048709 A	11-04-2000
WO 9526973 A	12-10-1995	US 5565323 A	15-10-1996
		US 5888498 A	30-03-1999
		AU 705230 B	20-05-1999
		AU 2204295 A	23-10-1995
		BR 9507241 A	16-09-1997
		CA 2186636 A	12-10-1995
		CN 1150433 A	21-05-1997
		EP 0751951 A	08-01-1997
		FI 963884 A	26-11-1996
		JP 9511398 T	18-11-1997
		NO 964073 A	29-11-1996
		NZ 283660 A	28-07-1998
		US 5760205 A	02-06-1998
		US 5840493 A	24-11-1998
		US 6027883 A	22-02-2000
		US 5976798 A	02-11-1999
WO 9817826 A	30-04-1998	US 5840493 A	24-11-1998
		AU 4912397 A	15-05-1998
		EP 0939832 A	08-09-1999